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JOSEPH ROSENBAUM
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H. H. Whetzel and I. E. Melhus

"He not only had a great many friends but he knew it too. He showed his affection for people so naively that one couldn't help returning it." No finer expression of the personal character of Joe Rosenbaum can be offered than these words of his stricken wife announcing the death of our young friend and colleague. We called him "Joe" because we sensed the human friendliness of him. We esteemed him for his enthusiasm and devotion in the labors of his profession. His researches in the field of plant pathology, to which the earlier years of his professional life were devoted, commanded our confidence and respect.

These characters of the man were already clearly defined in the days when, as a graduate student, he worked with the senior author in the laboratories of plant pathology at Cornell University. Keen and alert, he worked with the joyous enthusiasm, the clear thinking and the friendly devotion which every teacher recognizes as the mark of the promising student. With no trace of personal selfishness, which often mars the promises of a brilliant mind, he was an ideal assistant and companion in scientific endeavor. It was this rare combination of enthusiasm, personal charm and ability which during the later years of his short life made him a devoted and successful teacher of young men preparing to enter college. The last time I saw him he told me, with that generous pride of the successful teacher, of the high standing of the pupils from his laboratory in the entrance examinations in botany and biology. With undiminished enthusiasm and energy he had passed from the field of research to the more difficult and exacting realms of the successful teacher. And so, unsuspecting, I grasped his hand for the last time, and he left me as he had come to me in his college days, the kindly, able and enthusiastic Joe.

The junior author was fortunate in being associated with Joe as a fellow investigator when he served in the Bureau of Plant Industry, U. S. Department of Agriculture. In that association the sterling qualities of the man

gave greatest expression. He was ever ready to do his part in the fullest measure; and his enthusiasm, determination and untiring efforts, coupled with a keen analysis of his task, were stimulating and contributed much to lightening our labors in the field of potato disease investigations. His learning, alertness, openmindedness and honesty of scientific purpose made for implicit confidence in his interpretations and conclusions. In a word, his scientific contributions bear the stamp of a task well done.

The close contact of an isolated field station served to bring out those qualities of companionship so characteristic of the man and to emphasize the beautiful simplicity of his ideals in life. Here was shown his home life, presided over by his wife, a noble woman, radiating physical well-being, hope, and gladness in their path. Joe often spoke of his desire to have a small country place surrounded by plants in their natural beauty. His keen pleasure in serving others, his unselfishness, love of home simplicity, and his innate idealism are attributes that claim our homage.

Joseph Rosenbaum was born December 19, 1887, in Russia. He was descended from a long line of able Jewish Rabbis. He came with his father and the family to the United States, where they settled in Woodbine, New Jersey, in 1896. Here he received his grammar and high-school education. He entered the Massachusetts Agricultural College in 1906, transferring to the New York State College of Agriculture at Ithaca in 1907. He received the degree of B.S.A. from the latter institution in 1911. He supported himself while an undergraduate by writing and by miscellaneous work during summer vacation and through the school year. He received the degree of Doctor of Philosophy at Cornell University in 1914, having specialized in the field of plant pathology. During these graduate years he was special agent for the U. S. Department of Agriculture on ginseng diseases and, in 1914–1915, instructor in plant physiology in Cornell University. In 1915 he became a resident member of the staff of the Bureau of Plant Industry in the U. S. Department of Agriculture, where he remained, devoting himself to research in plant pathology and mycology, until 1920. From 1920 until the time of his death he was head of the Department of Botany and Biology at the Milford Preparatory School, Milford, Connecticut. Here he soon became a successful and much loved teacher. He died June 5, 1925, of bacterial endocarditis, after a three months' illness. He left a widow and two young daughters.

Dr. Rosenbaum was a member of the Alpha Chapter of Sigma Xi, a member and fellow of the American Association for the Advancement of Science, a member of the American Phytopathological Society, and of the Botanical Society of Washington, D. C. For one whose years in productive research were so few, he made a notable number of contributions to science.

PUBLICATIONS OF JOSEPH ROSENBAUM

The diseases of ginseng and their control. U. S. Dept. of Agr. Bur. Pl. Ind. Bul. 250. 1912. (Co-author with H. H. Whetzel.)

Formalin treatment of seed-beds of ginseng. Special Crops. September, 1912.

Experiments on the control of the fiber-rot or "rust" of ginseng. Special Crops. September, 1912.

Infection experiments with *Thielavia basicola* on ginseng. Phytopath. **2**: 191–196. 1912.

Root rots of ginseng and their control. Special Crops. July, 1913.

Treatment of soils and roots for root rots. Special Crops. August, 1913.

Alternaria panax, the cause of a root rot of ginseng. Jour. Agr. Res. **5**: 181–184. 1915. (Co-author with C. I. Zinnsmeister.)

Pathogenicity and identity of *Sclerotinia libertiana* and *Sclerotinia smilacina* on ginseng. Jour. Agr. Res. **5**: 291–299. 1915.

Phytophthora disease of ginseng. N. Y. (Cornell) Agr. Exp. Sta. Bul. 363: 61–106. 1915.

Ginseng diseases and their control. U. S. Dept. Agr. Farmers' Bul. 736: 1–22. 1916. (Co-author with H. H. Whetzel, J. W. Brann, and J. A. McClintock.)

Spongospora subterranea and *Phoma tuberosa* on the Irish potato. Jour. Agr. Res. **7**: 213–254. 1916. (Co-author with I. E. Melhus and E. S. Schultz.)

Studies of the genus *Phytophthora*. Jour. Agr. Res. **8**: 233–276. 1917.

Studies of the genus *Phytophthora*. Proc. Nat. Acad. Sci. **3**: 159–163. 1917.

A new strain of *Rhizoctonia solani* on the potato. Jour. Agr. Res. **9**: 413–420. 1917. (Co-author with M. Shapovalov.)

Influence of temperature and precipitation on the black leg of potato. Jour. Agr. Res. **13**: 507–513. (Co-author with G. B. Ramsey.)

The origin and spread of tomato fruit rots in transit. Phytopath. **8**: 572–580. 1918.

Studies with *Macrosporium* from tomatoes. Phytopath. **10**: 9–21. 1920.

Correlation between size of the fruit and the resistance of the tomato skin to puncture and its relation to infection with *Macrosporium tomato* Cooke. Amer. Jour. Bot. **7**: 78–82. 1920. (Co-author with Charles E. Sando.)

Infection experiments on tomato with *Phytophthora terrestria* Sherb. and a hot water treatment of the fruit. Phytopath. **10**: 101–105. 1920.

A Macrosporium foot-rot of tomato. Phytopath. **10**: 415–422. 1920.

PHOMOPSIS JUNIPEROVORA AND CLOSELY RELATED STRAINS ON CONIFERS

GLENN GARDNER HAHN

INTRODUCTION

The blight and canker of nursery cedars, caused by *Phomopsis juniperovora* Hahn (5) and known as cedar blight, probably should be regarded as a Phomopsis disease of conifers for reasons set forth in this paper. Stewart (16) and Duggar made the first recorded observations on this disease in 1896–7. Typical *P. juniperovora* continues to be in certain parts of the United States a virulent parasite, causing great damage in coniferous nurseries and also injury in ornamental plantings. It is now found to attack a number of heretofore unreported hosts which may be as vigorously attacked as is *Juniperus virginiana* L. (6), the host upon which red cedar blight, as the disease was earlier known (7), was investigated (4). Phomopsis strains closely related to *P. juniperovora*, but perhaps specifically distinct, have also been found to cause disease in representatives of a number of genera of conifers. The present paper is a progress report outlining the results of investigations in the United States.

GEOGRAPHICAL DISTRIBUTION

Typical *Phomopsis juniperovora* is known to occur in States extending along the Atlantic seaboard from Connecticut to Florida; from southern Minnesota and Wisconsin to Alabama; it is not known to date to extend any farther west than eastern Nebraska and Kansas (6). The States as heretofore published (5) include Illinois, Iowa, Kansas, Minnesota, Nebraska, New York, Ohio, and Pennsylvania. To this list may now be added Alabama, Connecticut, District of Columbia, Florida, Kentucky, Maryland, Missouri, North Carolina, Tennessee, and Wisconsin.

Closely related strains which have been found on conifers also are reported from California, District of Columbia, North Carolina, Pennsylvania, and Washington.

NEW FIELD HOSTS

Phomopsis juniperovora was originally described as occurring naturally on *Juniperus virginiana* (5) and *J. scopulorum* Sarg.[1] Early artificial in-

[1] The nomenclature herein followed is that contained in the revised check list of Commercially Important Trees of the United States, Forest Service, U. S. Dept. Agr., Dec. 15, 1924, and for ornamental conifers in Standardized Plant Names, American Joint Committee of Horticulture Nomenclature, 1924.

oculations (4) also demonstrated the potential parasitism of the *Phomopsis* on *J. virginiana, J. scopulorum, J. lucayana* Britt. (not *J. barbadensis* L.), *J. pachyphloea* Torr., *J. communis* L., *J. communis* var. *montana* (*J. sibirica* Burgsd.), *J. horizontalis* Moench (*J. prostrata* Pers.), *Thuja occidentalis* L., *T. orientalis* L., *T. plicata* Don., (*T. gigantea* Nutt.), *Cupressus glabra* Sudworth.

Diagnoses of field specimens sent into the laboratory have since shown that, with the exception of *J. pachyphloea,* all the above-mentioned inoculation hosts are naturally infected with *Phomopsis juniperovora.* Typical *P. juniperovora* has also been found on *J. procumbens* Sieb., (*J. chinensis* var. *procumbens* Endl.); *J. chinensis* var. *pfitzeriana* Spaett.; *J. sabina* L.; *J. sabina* var. *tamariscifolia* Ait.; *Cupressus sempervirens* L.; *C. macrocarpa* Hartw.; *C. arizonica* Greene; *C. goveniana* Gord.; *C. benthami* Carr.; *C. funebris* Endl.; *Chamaecyparis obtusa* Sieb. and Zucc., (*Retinospora obtusa* Sieb. and Zucc.); *C. pisifera* Sieb. and Zucc.; *C. pisifera* var. *plumosa* Beiss., (*Retinospora plumosa* Veitch); *C. lawsoniana* Parl., (*Cupressus lawsoniana* Murr.)

The Phomopsis strains related to *Phomopsis juniperovora* have been found on *Juniperus procumbens* Sieb.; *Thujopsis dolobrata* Sieb. and Zucc., (Hiba arbor-vitae); *Cephalotaxus drupacea* Sieb. and Zucc., (Japanese plum-yew); *Taxus baccata fastigiata* Loud., (Irish yew); *T. cuspidata* Sieb. and Zucc., (Japanese yew); *Taxodium distichum* (Linn.) Rich., (bald cypress); *Sequoia washingtoniana* (Winsl.) Sudw., (big tree); *S. sempervirens* (Lamb.) Endl., (redwood); *Cryptomeria japonica* D. Don, (common *Cryptomeria*); *Abies homolepis* Sieb. and Zucc., (*A. brachyphylla* Maxim, Nikko fir); *Pseudotsuga taxifolia* (Poir.) Britt., (Pacific coast type or Green Douglas fir, *Pseudotsuga douglasii* Carr., and the Rocky Mountain type, or Blue Douglas fir, *Pseudotsuga glauca* Mayr.); *Tsuga canadensis* (Linn.) Carr., (eastern or Canada hemlock); *Larix europaea* D. C. (*L. decidua* Mill., European larch); and *L. leptolepis* Murr. (Japanese larch).[2]

CULTURAL CHARACTERS

The separation of the Phomopsis strains into the two arbitrary groups, namely the A group, definitely typical *P. juniperovora,* and the B group, which is composed of the closely related strains, is based in part on cultural behavior in preliminary tests. Lines of the first-named group, which were isolated and studied from *Juniperus virginiana, Cupressus arizonica, C. sempervirens, C. funebris,* and *Chamaecyparis obtusa,* differed somewhat in their reactions from lines from the B group studied from *Juniperus pro-*

[2] It will be noted that the 22 host species of typical *Phomopsis juniperovora* all belong to four genera of cedars; while the related strains are mostly from other coniferous hosts.

cumbens, Thujopsis dolobrata, Cephalotaxus drupacea, Taxus baccata fastigiata, Taxodium distichum, Sequoia sempervirens, Pseudotsuga taxifolia (both Pacific coast and Rocky Mountain types) and *Larix europaea.*

For the A group, tests were made with cultures from 15 localities in Maryland, District of Columbia, North Carolina, Illinois, and Missouri; 16 lines of this group were obtained from single spores. Cultures of the B group were likewise studied from 40 localities in Pennsylvania, District of Columbia, North Carolina, Illinois, Washington, and California; 45 lines were obtained from single spores.

Freshly isolated cultures of strains from the A group generally produce the characteristic empire-yellow to orange-rufous color and flaming orange crystals in the medium, both of which have been previously reported as diagnostic for *P. juniperovora* on prune or cornmeal agar (5). This orange color is also vividly produced in cornmeal plus 2 per cent cane sugar (saccharose) agar. Only one strain was found which produced it in glycerine agar. On Thaxter's medium, certain strains produced a wine color in the surface growth. On Molisch's medium the orange color appeared in the substratum, while the dark grayish surface growth had a delicate lilac cast with here and there shadings of green.

Growth of the strains from the B group on Thaxter's and Molisch's media, and on cornmeal, cornmeal plus 2 per cent cane sugar and glycerine agars, was unaccompanied by the production of the vivid orange-red color in the substratum described above. On Thaxter's medium a pale rose color appeared in the surface growth. On Molisch's medium the surface growth was indistinguishable from that of the cultures composing the A group. Growth in the B group generally was slightly more rapid, and pycnidia commenced to appear somewhat earlier than in the A group.

As the cultures of strains of both groups age, they become very much alike. Abundant pycnidiospores are produced by each group. The outstanding cultural characteristics which distinguished the two groups was the production of the orange-red color associated with the early growth of typical *Phomopsis juniperovora.* The production of this color also held for reisolations of strains of the A group from Douglas fir infected artificially with strains of *P. juniperovora* recently isolated from *Juniperus* and *Cupressus* from North Carolina and Maryland, respectively. Single spore cultures from red cedar were observed which produced only a bare suggestion of the color or lacked it entirely. These strains were classed as *P. juniperovora,* despite the abnormality in the color character, on the basis of their origin on a cedar host in a locality where the typical color-producing fungus was known to occur. Old cultures were observed to lose gradually their ability to reproduce the orange-red color. Continued investigation may demonstrate that color production is a variable characteristic. Heald

and Studhalter (9) found that different strains of *Strumella coryneoidea* Sacc. and Wint. in culture showed great variation in the amount and intensity of dark-brown or black coloration.

In line with the consideration of color in artificial culture as a variable factor, Bottomley (2), in culturing a highly parasitic *Phomopsis* causing blight of seedling *Cupressus* (*C. arizonica, C. macrocarpa,* and *C. torulosa* D. Don) in South Africa, did not obtain the orange-red coloration descriptive of cultures of *P. juniperovora* isolated from *Cupressus* spp. in the United States. She describes only a greyish-green color and the spores oozing from pycnidia in a pale salmon-pink drop.

MORPHOLOGICAL CHARACTERS

With regard to the size and shape of their spores, the closely related strains of *Phomopsis* differ only slightly from *P. juniperovora* as earlier described (5). The A and B groups both produced the two types of spores known as α and β spores respectively in the same or different pycnidia both naturally and under cultural conditions. The α spores were shorter, being usually oblong to ellipsoid, or spindle shaped with subacute extremities generally biguttulate; whereas the β spores were longer, filamentous, curved, sickle, S-shaped, or hooked at the upper end (5). The measurements of the α spores of the Transvaal fungus, as given by Bottomley (2), differ only very slightly in shape and size from *P. juniperovora.* The question therefore comes up as to what influence the particular host may have on the size of the pycnidiospores. While Shear, Stevens and Wilcox (15) found constant uniformity for the cultural characteristics and size of ascospores for *Physalospora malorum* and *Botryospheria ribes,* at the same time they admit fluctuation in size of pycnidiospores from different hosts. These writers call attention to Paddock's (10) observation with regard to the variation of the average size of spores of *Sphaeropsis malorum* produced on different hosts.

By reason of the slight differences between the two groups, the Phomopsis strains on conifers, other than those included in group A, may be tentatively regarded as strains of *P. juniperovora;* but, for the sake of clearness, the two groups are kept distinct throughout this report. An extensive cultural and morphological comparison between *P. juniperovora* and the related strains for the definite determination of the latter is now being undertaken, together with an effort to develop a perfect stage. These data will be reported later. The results of preliminary artificial inoculation experiments with strains from both groups are given in the following section.

NEW HOSTS ESTABLISHED BY ARTIFICAL INOCULATION

Cross inoculation experiments have indicated physiological relationships between strains of *Phomopsis juniperovora* and closely related strains of

the B group. Douglas fir, *Pseudotsuga taxifolia* commonly called Pacific Coast, Oregon or Green Douglas fir, has been shown (Table 1) to be a common host under artificial conditions, when inoculated with strains of *P. juniperovora* isolated from *Juniperus virginiana, Cupressus sempervirens, C. arizonica, Chamaecyparis obtusa,* and related strains isolated from *Taxus baccata fastigiata, Taxodium distichum,* and *Pseudotsuga taxifolia,* Pacific coast type. Results of cross inoculation of Phomopsis strains on various other hosts are presented in tables 2 and 3 and in the discussion.

METHODS OF INOCULATION

Artificial inoculations were made from April to July by wounding host plants from two to fourteen years old. At this time the plants were all growing actively. The parts to be inoculated were first thoroughly cleansed with a cotton swab and 95 per cent alcohol. A bit of a culture of *Phomopsis* in the pathological greenhouse where the plants were kept in an iceless bark with a sterile scalpel. The inoculations were protected by being wrapped with raffia and moist cotton. Some of the inoculations were made in the pathological greenhouse where the plants were kept in an iceless refrigerator[3] for 48 hours after inoculation; others were made in the plant beds out of doors. The latter received only the routine watering which is given the out-of-door plants. In all tests control wounds which were made on trees inoculated with the fungus or on uninoculated trees were treated as just described, with this exception—a bit of sterile cornmeal agar was inserted instead of the mycelium growing on agar.

RESULTS OF INOCULATIONS

Infections in young tissue as the result of successful inoculations with *Phomopsis juniperovora* were commonly found to show signs of spreading within five to ten days after the insertion of the inoculum. A typical terminal or lateral blight resulted. Infections obtained on laterals may extend down into the main axis. On stems of larger diameter, cankers formed as the result of the fungous extension, which grew slowly, generally involving only one side of the branch or main axis and killing one or more laterals on that side. Such body cankers which did not become confluent usually became more or less limited and tended to callus over. Cankers of this type showed a definite separating fissure extending into the inner bark along the periphery of the canker. Below this cracking of the cortical tissues callus tissue formed. The pathological aspects of the blight and canker as just described are very similar to those stated by Wilson (17, 18, 19, 20,

[3] Hunt, N. R. The iceless refrigerator as an inoculation chamber. Phytopath. **9**: 211–212. 1919.

TABLE 1.—*Results of inoculation experiments with strains of typical Phomopsis juniperovora and closely related strains on Pseudotsuga taxifolia*

Host	Age of host in yrs.	Location of plants	*Phomopsis juniperovora*		Inoculations		Controls		Age of wood in years at point of infection
			Strains of A group	Source of culture	Number	Percentage of infection	Number	Percentage of infection	
Pseudotsuga taxifolia. tree No. 4	8	greenhouse	*Juniperus virginiana* 41369 C	single spore	12	100			1, 2, 3, 5
do 6	8	do					9	0	
do 20	9	outside	do	do	6	83	2	0	3, 4, 5, 7
do 1	8	greenhouse	*Cupressus sempervirens* 41372 C	spore horn	6	100			2, 3, 4
do 2	8	do	do	do	9	100			1, 2, 3, 4, 5
do 3	8	do					6	0	
do 21	14	outside	do	do	11	100	5	0	2, 3, 4, 5, 6, 8, 9
do 6	8	greenhouse	*Cupressus arizonica* 41041 A	single spore	12	100	4	0	1, 2, 3, 4, 5
do 8	8	do	*Pseudotsuga taxifolia* 41048 A. Reisolation from inoc. with *Cupressus* 41041 A	inner bark	12	100	5	0	1, 2, 3, 4, 5, 7
do 11	8	do	*Chamaecyparis obtusa* 41376 B	do	9	100	2	0	1, 2, 3, 4
Totals (8 plants inoculated)					77	99	33	0	

TABLE 1.—(*Continued*)

Host	Age of host in yrs.	Location of plants	*Phomopsis juniperovora*		Inoculations		Controls		Age of wood in years at point of infection
			Strains of B group	Source of culture	Number	Percentage of infection	Number	Percentage of infection	
tree No. 7	8	greenhouse	*Taxus baccata fastigiata* 41035 A	spore horn	6	83	5	0	2, 4, 6
do 8	8	do					9	0	
do 12	8	do	*T. baccata fastigiata* 41037 A	single spore	11	46	5	0	1, 2, 3
do 13	8	do	*Taxodium distichum* 41040 A	do	10	50	4	0	2, 3, 4, 5, 6
do 10	8	do	*Pseudotsuga taxifolia* 41039 A	do	7	29	5	0	3, 5
do 22	14	outside	do	do	11	64	5	0	2, 3, 4, 5, 6
do 14	8	greenhouse	*P. taxifolia* 41045 A	do	12	17	4	0	5, 7
Totals (6 plants inoculated)					57	46	37	0	
TOTALS	3 trees inoculated and 3 checks outside				28	82	12	0	
	11 trees inoculated and 11 checks in greenhouse				106	74	58	0	

21, 22) and Alcock (1) for a serious Phomopsis disease on Douglas fir, hemlock, and Japanese larch in Great Britain which is attributed to *Phomopsis pseudotsugae* Wilson.

The fungus appears to spread most rapidly longitudinally in the inner bark, killing the cambium and staining the wood a brownish color. Very small stems, less than one-half inch in diameter, are quickly girdled, the parts beyond the point of inoculation dying more rapidly than parts below. The lateral extension of the parasite in stems of one-half or more inches in diameter is very much slower. In the latter instance the outer bark apparently offers considerable mechanical resistance to the inroads of the fungus.

The control wound in no case showed signs of infection but promptly healed over. An additional control was provided for the experiments by the inoculations of *Pestalozzia sp.*, a fungus which occurs commonly and abundantly on dead nursery cedars and conifers generally. Eight inoculations with the mycelium of *Pestalozzia sp.* made according to the method just described for *P. juniperovora* and related strains, and four controls made in the same manner on *Cupressus arizonica* remained free from disease. These results confirm those obtained by Bottomley (2) with a *Pestalozzia* sp. on the same host in Transvaal, South Africa.

The results of the inoculations on *Pseudotsuga taxifolia* are summarized in table 1. As has been stated above, Douglas fir is a common host for all the strains tested, namely those isolated from *Juniperus, Cupressus, Chamaecyparis, Taxus, Taxodium,* and *Pseudotsuga.* The symptoms of the disease produced, whether it was on young twigs or on older parts, was identical in all cases. The strains of typical *Phomopsis juniperovora* appear to be more virulent than the strains of the B group.

The foregoing inoculations on *Pseudotsuga taxifolia,* Pacific coast form, do not agree with the negative result published for *P. juniperovora* on this host in 1920 (5). This early negative inoculation consisted of a single test made in January in the greenhouse on a dormant tree of Douglas fir, of the Rocky Mountain type. This tree was the only one available for study at the time.

The results of inoculation experiments on two-year-old *Cupressus arizonica* are stated in table 2. The plants used in these experiments were exceptionally vigorous. Strains of *P. juniperovora* from *Juniperus, Cupressus,* and *Chamaecyparis* were extremely parasitic upon this host. Inoculation results with *P. juniperovora* isolated from *Cupressus* parallel those obtained by Bottomley (2) with the Transvaal *Phomopsis* upon the same host. Closely related Phomopsis strains from *Taxus* and Douglas fir produced negative results even when inoculated in very young tissue of *C. arizonica.*

The results of inoculation experiments with strains of the A and B groups on *Thuja orientalis* are stated in table 3. Strains from *Juniperus,*

Cupressus, and *Chamaecyparis* proved to be highly virulent on this host; Phomopsis strains from *Taxus* and Douglas fir failed to infect either very young or older tissues.

Three-year-old plants of *Taxodium distichum* were inoculated with strains of *Phomopsis juniperovora* from *Juniperus* and *Cupressus* (group A) and a Phomopsis strain from *Taxus* (group B). The strains of the A group were able to infect one- and two-year-old wood of this host; the infections on three-year old wood became limited and healed over. Of the 30 inoculations with strains of *P. juniperovora*, 22 were positive, while 6 inoculations with a Phomopsis strain from *Taxus* produced negative results. In all cases checks remained uninfected and promptly healed over.

A single three-year-old plant of *Larix europaea* inoculated with *Phomopsis juniperovora* showed infection within three days after inoculation.

TABLE 2.—*Results of inoculation experiments with strains of Phomopsis juniperovora and closely related strains on two-year old Cupressus arizonica in the greenhouse*

Host	No. of plants	*Phomopsis juniperovora*		Inoculations		Controls[a]	
		Strains of A group	Source of culture	Number	Per cent infected	Number	Per cent infected
Cupressus arizonica	3	*Juniperus virginiana* 41369C	single spore	11	100	4	0
	2	*Cupressus sempervirens* 41372C	spore horn	6	100	4	0
	2	*Chamaecyparis obtusa* 41376C	inner bark	6	100	4	0
	2	*P. taxifolia* 41048A. Reisolation from inoculation with *Cupressus* 41041A	inner bark	7	100	3	0
Totals	9			30	100	15	0
		Strains of B group					
Cupressus arizonica	3	*Taxus baccata fastigiata* 41035A	spore horn	9	0	3	0
	3	*T. baccata fastigiata* 41047A	single spore	9	0	4	0
	2	*Pseudotsuga taxifolia* 41045A	do	6	0	3	0
Totals	8			24	0	10	0

[a] Control inoculations were made on 7 separate plants.

All the inoculations on this plant were positive; and three checks on the same plant remained uninfected.

Negative inoculation results were obtained with strains of the A, or typical *Phomopsis juniperovora*, group on the following:

Juniperus stricta, 6 inoculations with a strain from *Juniperus* on 2 two-year- old plants.

Picea excelsa, 29 inoculations with a strain from *Juniperus* on 5 young plants; 8 inoculations with a strain from *Cupressus* on an eight-year-old plant.

Pinus radiata, 6 inoculations with a strain from *Juniperus* on 2 two-year-old plants; 4 inoculations with a strain from *Cupressus* on 2 two-year-old plants.

TABLE 3.—*Results of inoculation experiments with strains of Phomopsis juniperovora and closely related strains on two-year old Thuja orientalis in the greenhouse*

Host	No. of plants	*Phomopsis juniperovora*		Inoculations		Controls[a]	
		Strains of A group	Source of culture	Number	Per cent infected	Number	Per cent infected
Thuja orientalis	3	*Juniperus virginiana* 41369C	single spore	15	100	5	0
	4	*Cupressus sempervirens* 41372C	spore horn	15	100	10	0
	2	*Chamaecyparis obtusa* 41376C	inner bark	8	100	4	0
	2	*P. taxifolia* 41048A. Reisolation from inoculation with *Cupressus arizonica* 41041A	inner bark	12	100	4	0
Totals	11			50	100	23	0
		Strains of B group					
Thuja orientalis	3	*Taxus baccata fastigiata* 41035A	spore horn	9	0	4	0
	2	*T. baccata fastigiata* 41037A	single spore	8	0	3	0
	2	*Pseudotsuga taxifolia* 41045A	single spore	12	0	4	0
Totals	7			29	0	11	0

[a] Control inoculations were made on 8 separate plants.

P. strobus, 10 inoculations with a strain from *Juniperus* on 2 four-year-old plants; 14 inoculations with a strain from *Cupressus* on 3 three-year-old plants.

P. excelsa, 18 inoculations with a strain from *Juniperus* on 3 five-year-old plants.

P. sylvestris, 12 inoculations on the young growth of 2 nine-year old plants.

Negative inoculation results were also obtained with a strain of the B group from *Taxus* on *Picea excelsa.* Twenty-five inoculations on 4 young plants were negative.

The above-listed negative results for *Phomopsis juniperovora* on *Pinus* and *Picea* parallel other negative results (5) previously obtained on *Picea parryana* (Andre) Parry, *P. engelmanni* Engelm., *Pinus edulis* Engelm., *P. glabra* Walt., and *P. taeda* Linn.

FACTORS PREDISPOSING TO PHOMOPSIS ATTACK

Young coniferous nursery stock is virulently attacked by the cedar-blight organism, *Phomopsis juniperovora.* Field observations have shown that it is fully capable of entering and attacking thoroughly healthy trees unweakened by other causes. This ability to infect readily young, tender, unwounded tissue which has been demonstrated by Bottomley (2) in her experiments with a *Phomopsis sp.* helps to explain the appearance of typical cankers on older stock, caused by *P. juniperovora* and closely related Phomopsis strains getting into the older tissue of the main stem through the infected side laterals (Plate LII).

Contributory evidence of the parasitic ability of *P. juniperovora* as a nursery-seedling parasite has been brought out by Rathbun-Gravatt in her inoculation experiments with damping-off fungi. In one experiment she showed that *P. juniperovora* caused root-rot of pine seedlings (11). She not only found that this fungus caused considerable decay of coniferous tap roots (12), but also in direct inoculation of coniferous stems evidenced parasitic ability (13). *P. juniperovora,* out of six miscellaneous fungi tested, including *Pestalozzia sp.,* a species commonly associated with the *Phomopsis,* was the only one which caused any radicle decay. It also appeared to cause a decrease in sprouting (14). Hartley (8) and his associates in soil inoculations previously found that *P. juniperovora* did not cause damping-off of pine seedlings. The possible reason for this may be the inability of the fungus to maintain itself in the soil saprophytically until the seed has sprouted.

During growing seasons when excessive moisture is present the organism becomes particularly aggressive, blighting entire beds of seedling stock in a very short period of time. In one nursery where the *Phomopsis* has

caused heavy damage for many years it was found that overhead irrigation greatly increased loss due to the blight. A discontinuance of this system

FIG. 1. *Pseudotsuga taxifolia*, Pacific Coast type (FP 41045) affected with a Phomopsis strain which closely resembles *Phomopsis juniperovora* and which causes a terminal and lateral blight of 3-year old nursery seedlings. Natural size.

of irrigation aided appreciably in the control of the disease. The crowding of plants in the seedling beds, and heavy shading also increased the toll of the disease, making possible moist chamber conditions for the abundant propagation of the fungus and rapid dissemination from plant to plant.

Since there is now abundant experimental evidence that infection takes place through wounds, injuries to the trees, no matter how produced, must be looked upon as predisposing factors. Transplanting, cultivating, pruning, in fact mechanical wounds of all sorts may lead to infection by the organism. It is not known how much insect injuries have to do with the spread of *P. juniperovora*. In the grafting process wherein scion stock of different horticultural varieties of cedars is used on rootstocks of *Juniperus virginiana*, heavy loss from the blight has been experienced, probably due to the fact that the fungus may enter through the graft union.

Undoubtedly the weakening of older trees by unfavorable growth conditions is likewise a factor for consideration in the spread of blight and canker. Ornamental trees, some of considerable age, have been found infected with *P. juniperovora* and strains of *Phomopsis*. Cankers from such trees placed in moist chamber produced Phomopsis spore tendrils in great abundance (Plate LIII).

EXTENT OF THE DAMAGE

Throughout the Middle West the growing of grafted and seedling nursery stock of *Juniperus virginiana*, especially the Platte River type, is, in certain seasons, seriously affected. A number of nurseries have been forced out of cedar growing by the blight. In Alabama the southern type of red cedar, *Juniperus lucayana*, is reported to be heavily infected. In coniferous nurseries in the South where conifer propagation is rapidly increasing, the successful propagation of ornamental cedars, particularly *Cupressus* and *Juniperus*, is extremely difficult during certain seasons because of damage by *Phomopsis*. Bottomley (2) has reported the devastating effect of a closely related Phomopsis blight in coniferous nurseries during certain years in Transvaal, South Africa.

The injury caused by *Phomopsis juniperovora* and the closely related strains commonly occurs in ornamental plantings in private gardens, plantings about public buildings, and city parks. The disease frequently produces an unsightly effect in these plantings due to the dead twigs and branches which appear among the healthy green ones. Evans (3) reports a dying-off disease of *Cupressus macrocarpa* hedges in South Africa due to a *Phomopsis sp.*

Two localities are known so far where strains of *Phomopsis* have affected naturally seedlings of Douglas fir, *Pseudotsuga taxifolia*, Pacific coast type, growing in nurseries, namely, North Carolina and Stabler, Wash-

ington. In North Carolina approximately 75 per cent loss occurred among three-year-old nursery stock. Tendrils of *Phomopsis* appeared abundantly upon the blighted plant parts. Microscopic examination showed both α and β spores present. The injury consisted of a blighting or death of the terminal growth (Fig. 1) or the entire plant was involved (Plate LIV), the disease apparently entering the main stem through a short lateral. Where the leader had been killed, a lateral usually took its place (Fig. 1). Infected seedlings were observed to be smaller than healthy ones of the same age. At Stabler, Wash., the blight affected both low and high altitude forms, apparently causing a greater damage among the former.

A Phomopsis strain was also found in Pennsylvania associated with a twig blight of 15-year-old Douglas fir, Rocky Mountain form. This damage was slight as compared with that observed in the infected nursery stock of the Pacific coast form just referred to. In the North Carolina locality it was noted that seedlings of the Rocky Mountain form growing close by the Pacific coast form were uninfected. Wilson (17,18) describes *P. pseudotsugae* as causing damage on the Green Douglas fir, *Pseudotsuga douglasii* Carr. (*P. taxifolia*, Pacific coast type). He also states that the disease has been occasionally found on *P. glauca* Mayr, Blue Douglas fir (*P. taxifolia*, Rocky Mountain type), and the variety *caesia* of *P. douglasii* (22). A recent communication from Dr. J. S. Boyce, who has made a survey of the Phomopsis disease of conifers caused by *P. pseudotsugae*, states, "*P. pseudotsugae* rarely attacks Blue Douglas fir in England or Scotland, which is also true on the Island of Bornholm in Denmark."

Our knowledge of the extent of the damage due to cedar blight in nurseries has in the past been somewhat confused due to the fact that disease caused by *Phomopsis* formerly has been attributed to frost, heat, drought, or transplant injury. Wilson (22) described a somewhat similar situation for the Phomopsis disease of conifers in the British Isles.

SUMMARY

Phomopsis juniperovora, the fungus causing cedar blight, is now known to be a widespread parasite in the United States causing serious damage in coniferous nurseries and ornamental plantings. Closely related Phomopsis strains have also been found which cause a blight and canker whose symptoms are similar to those of cedar blight. These strains, which have been placed arbitrarily in a B group, show certain cultural differences from the strains of the typical *P. juniperovora*, or A group. For reasons stated in the foregoing progress report the members of the B group may be regarded tentatively as strains of *P. juniperovora*, but for the sake of clearness they are considered separately. Twenty-two hosts, 12 of these new, are reported for the A group or *Phomopsis juniperovora*, together with 13 additional

hosts for the related strains. The A group contains the strains which are known to be definitely *Phomopsis juniperovora* isolated from the following genera: *Juniperus, Cupressus, Thuja,* and *Chamaecyparis;* the B group, those from *Juniperus, Thujopsis, Cephalotaxus, Taxus, Taxodium, Sequoia, Pseudotsuga,* and *Larix.* Host species of the above genera are listed together with species of *Abies, Cryptomeria,* and *Tsuga* on which *Phomopsis* was found but not studied in culture.

Preliminary spore measurements of strains of *Phomopsis juniperovora* and related Phomopsis strains show slight differences in the size and shape of their α and β spores, both of which are produced in abundance by both groups.

Host relationships for *Phomopsis juniperovora* and closely related strains have been established by preliminary artificial inoculations. Strains of both the A or typical *P. juniperovora* group and the related B group are parasitic on eight-year-old trees of *Pseudotsuga taxifolia,* Pacific coast form. Strains of the former group appear to be most virulent. Strains of the *P. juniperovora* group from *Juniperus, Cupressus,* and *Chamaecyparis* were also very parasitic on two-year-old stock of *Cupressus arizonica* and *Thuja orientalis*; negative results were obtained on these two last-named hosts with strains of the B group from *Taxus* and *Pseudotsuga.* Positive results were obtained on *Larix europaea* with *P. juniperovora* from *Juniperus.* Negative results were obtained on both *Pinus* spp. and *Picea excelsa* with strains of the two groups.

The predisposing factors to cedar blight and canker caused by *Phomopsis juniperovora* and to the similar blight and canker caused by the closely related Phomopsis strains are discussed, together with the extent of the damage associated with the disease.

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LITERATURE CITED

1. ALCOCK, N. L. A Phomopsis disease of Douglas fir. Gard. Chron. **69**: 45. 1921.
2. BOTTOMLEY, A. M. A preliminary investigation into a disease attacking young Cupressus plants. South African Jour. Sci. **15**: 613–617. 1919.
3. EVANS, I. B. POLE. Report of the botanist. VII. Pathological. Union South Africa Dept. Agr. Rept. **1918–19**: 77–79. 1920.
4. HAHN, G. G., C. HARTLEY, and R. G. PIERCE. A nursery blight of cedars. Jour. Agr. Res. **10**: 533–540. 1917.
5. ———. *Phomopsis juniperovora,* a new species causing blight of nursery cedars. Phytopath. **10**: 249–253. 1920.

6. ————————. Cedar blight caused by *Phomopsis juniperovora* Hahn. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reporter, Suppl. 42. 316–318. 1925. [Mimeographed.]
7. HARTLEY, C. The blights of coniferous nursery stock. U. S. Dept. Agr. Bul. 44. 1913.
8. ————————, T. C. MERRILL, and A. S. RHOADS. Seedling diseases of conifers. Jour. Agr. Res. **15**: 521–558. 1918.
9. HEALD, F. D., and R. A. STUDHALTER. The Strumella disease of oak and chestnut trees. Penn. Dept. Forest. Bul. 10. 1914.
10. PADDOCK, W. The New York apple-tree canker. N. Y. (Geneva) Agr. Exp. Sta. Bul. 163: 179–206. 1899.
11. RATHBUN, A. E. Root rot of pine seedlings. Phytopath. **12**: 213–220. 1922.
12. ————————. Damping-off of taproots of conifers. Phytopath. **13**: 385–391. 1923.
13. RATHBUN-GRAVATT, A. Direct inoculation of coniferous stems with damping-off fungi. Jour. Agr. Res. **30**: 327–339. 1925.
14. ————————. Germination loss of coniferous seeds due to parasites. 1925. [Unpublished manuscript.]
15. SHEAR, C. L., N. E. STEVENS, and M. S. WILCOX. *Botryosphaeria* and *Physalospora* in the eastern United States. Mycologia **17**: 98–107. 1925.
16. STEWART, F. C. The Phoma blight of red cedar. Phytopath. **8**: 33–34. 1918.
17. WILSON, M. A new disease of the Douglas fir in Scotland. Trans. Roy. Scottish Arb. Soc. **34**: 145–148. 1920.
18. ————————. A new species of *Phomopsis* parasitic on the Douglas fir. Trans. and Proc. Bot. Soc. Edinburgh (1919–20) **28**: 47–49. 1920.
19. ————————. A newly-recorded disease on Japanese larch, caused by *Phomopsis pseudotsugae*. Trans. Roy. Scottish Arb. Soc. **35**: 73–74. 1921.
20. ————————. The diseases of the Douglas fir. Trans. Roy. Scottish Arb. Soc. **35**: 77–78. 1921.
21. ————————. The occurrence of the Phomopsis disease of the Japanese larch in Yorkshire. Trans. Roy. Scottish Arb. Soc. **36**: 115–116. 1922.
22. ————————. The Phomopsis disease of conifers. Great Britain Forest. Comm. Bul. 6. 1925.

DESCRIPTION OF PLATES

PLATE LII

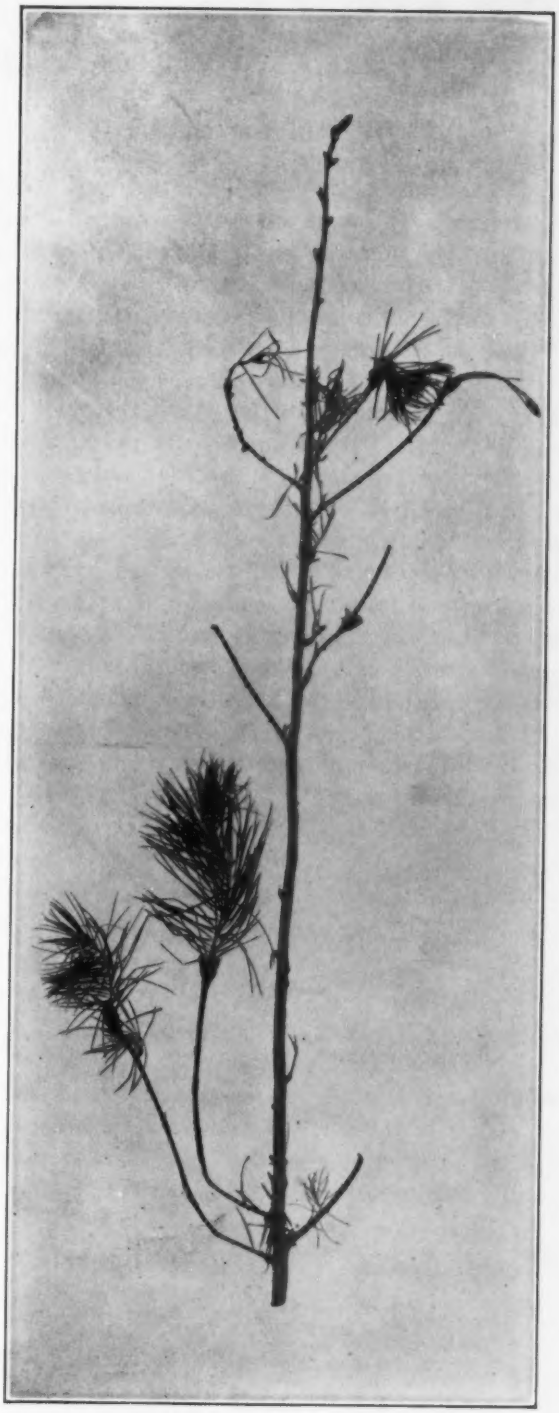
Juniperus virginiana affected with *Phomopsis juniperovora* (FP 41046). The canker on a 2-year old nursery seedling has girdled the main stem. Pustules abundant. Approximately 1½ times natural size.

PLATE LIII

Phomopsis strain closely resembling *P. juniperovora* on *Taxus baccata fastigiata* (FP 41035). Moist chambered limbs of large infected tree showing spore tendrils of *Phomopsis*. Natural size.

PLATE LIV

Pseudotsuga taxifolia, Pacific Coast type (FP 41045) affected with a Phomopsis strain which closely resembles *Phomopsis juniperovora*. The entire terminal down to the base of the 3-year old nursery seedling was blighted. Spore tendrils of *Phomopsis* appeared on the stems in moist chamber. ½ natural size.



A NEW BACTERIAL DISEASE OF THE BEAN

WALTER H. BURKHOLDER

During the last few years in New York State the diseases[1] of bean (*Phaseolus vulgaris* L.) commonly referred to as the bacterial blights have been very severe, and have been the chief limiting factor in the production of such varieties as the Red Kidney. Many acres of this variety have gone unharvested owing to the ravages of these diseases. That the severity and the rapid spread of the bacterial blights during recent years were due to peculiar weather conditions seemed scarcely feasible. It was thought formerly, and with very good reason, that the blights were favored by hot weather. The weather in New York State during these recent severe epiphytoties has been varied but, for the most part, cool. That certain very virulent strains of *Phytomonas phaseoli* and *Phyt. flaccumfaciens* occurred and had spread over the State did seem possible. For this reason, late in the season of 1924 many specimens of the disease were collected from various parts of the State, and isolations of the causal organism attempted. Some surprise was experienced when, in many cases, it was impossible to obtain a yellow bacterium from badly diseased plants. However, a pale, colorless to white bacterium always appeared in these instances, and not infrequently was found associated with *Phyt. phaseoli* and *Phyt. flaccumfaciens*. The first few plates of the organism were discarded as contaminated, but, upon continued isolation, inoculation experiments were decided upon. These first experiments on some 16 Red Kidney plants immediately proved the organism to be pathogenic and quite virulent. This led to a careful observation of the disease in the field and in the greenhouse, and to a study of the pathogene in the laboratory.

SYMPTOMS

The symptoms produced on the bean plant by the pathogene under discussion are in the majority of cases not dissimilar to those produced by *Phyt. phaseoli* and *Phyt. flaccumfaciens*. In fact it is frequently impossible to distinguish the three diseases in the field. For this reason it has appeared best, when talking to growers, to use the term bacterial blight to cover all these troubles of the crop. The symptoms of this new disease are as follows.

[1] *Phytomonas phaseoli* E. F. S. and *Phyt. flaccumfaciens* Hedges cause similar diseases of the bean in New York State.

Wilt. A wilt of a portion or of the entire plant may occur, similar to that described by the writer[2] due to *Phyt. phaseoli* and by Hedges[3] due to *Phyt. flaccumfaciens.* The leaves wilt, turn brown and cling to the plant. Young pods may wither and fail to produce seed. In young plants the stalk dries, becomes moderately thin and topples over. When seedlings are artificially inoculated in the greenhouse by pricking the stem, a wilt and death of the entire plant may ensue within two weeks. This seedling wilt, however, has seldom been observed in the field. A wilt of the mature plant in the field, on the other hand, is one of the commonest symptoms. The disease in this form may spread over an entire field of Red Kidneys in a very short time. This wilt, as well as that caused by the other organisms, is the result of the pathogene infecting the vascular system.

At other times when the bacteria have gained entrance to the vessels and conditions are not favorable for a wilting to occur, a distinct dwarfing of the plant takes place.

On the leaves. Probably the most characteristic symptom of this bacterial disease occurs on the leaves of plants affected with the dwarfing described above, or on other systemically infected plants showing slight wilting symptoms. The younger and top leaves of the plant become mottled with pale and dark green areas (Fig. 1). As a rule the darker areas are closer to the large veins, and the lighter green areas are between. A crinkling of the leaf also occurs. This condition may cover an entire leaf or take place to a less degree in spots. This mottling has been found frequently in Red Kidney fields and has been mistaken by growers for mosaic, which at times it closely resembles. On the White Marrow this symptom even more closely resembles mosaic, but can usually be distinguished by the presence of other symptoms of this bacterial disease.

Necrotic lesions also occur on the bean leaves (Fig. 2). These spots vary in size, from one-half inch in diameter to areas covering the entire leaflet. They may be green and wilted at first, but later dry out and become brown. Frequently they have a light yellow-green border. The writer has been unable to distinguish them from similar spots caused by *Phyt. phaseoli* and *Phyt. flaccumfaciens.*

On the stems. Reddish, necrotic lesions extending longitudinally along the stem have been observed on plants affected with this disease. These lesions may be due to local infections, or may develop from the bacteria breaking through to the surface in vascular infections. In the latter case, small gray viscid drops have been observed on the lesions. Upon micro-

[2] Burkholder, Walter H. The bacterial blight of the bean: a systemic disease. Phytopath. **11**: 61–69. 1921.

[3] Hedges, Florence. Bacterial wilt of beans (*Bacterium flaccumfaciens* Hedges) including comparisons with *Bacterium phaseoli.* Phytopath. **16**: 1–22. 1926.

scopic examination these have been found to be masses of bacteria, and are not unlike those occurring on fire blight lesions of apple and pear trees.

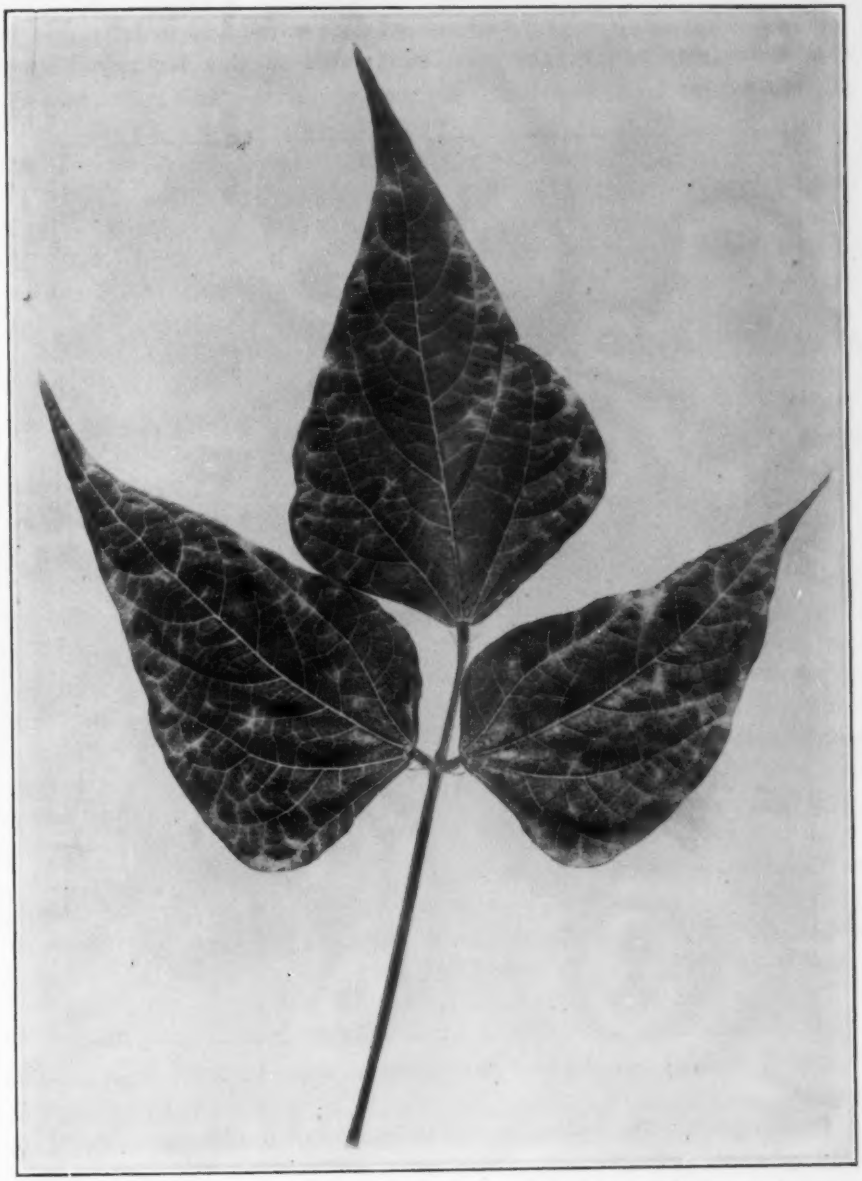


FIG. 1. Leaf of a White Marrow plant showing mottling due to the organism in the vascular system.

When bean plants are inoculated at the cotyledon or leaf nodes by the introduction of the pathogene on the point of a needle, a stem girdle not infrequently results. In appearance this symptom is identical with that due to *Phyt. phaseoli.* No work has been done in the field to determine the extent or frequency of the stem girdle due to this recently isolated bacterium.

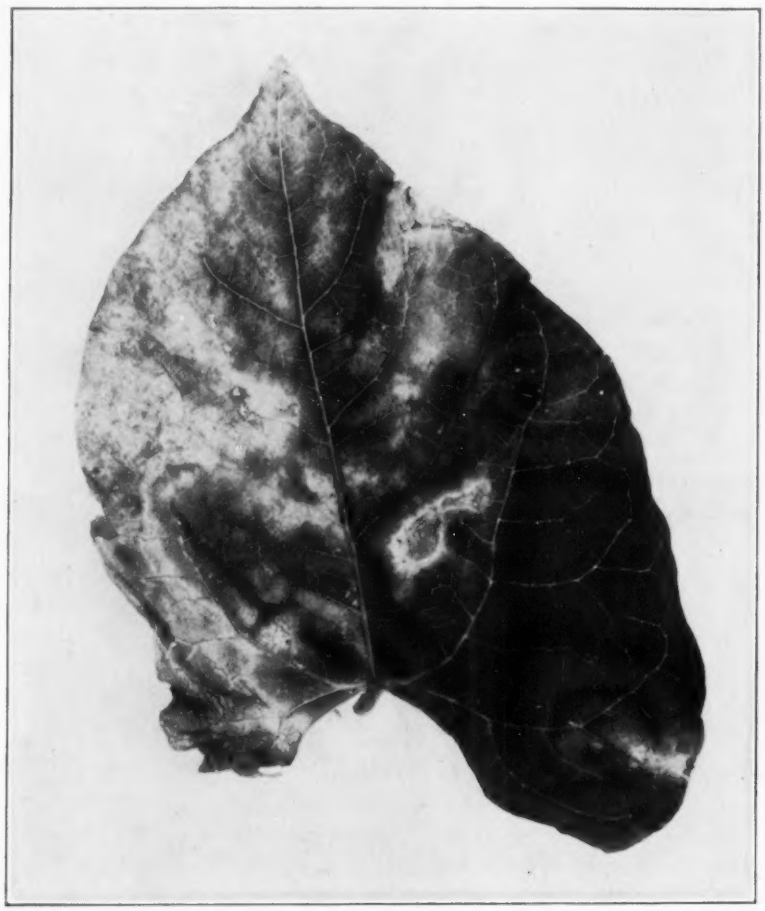


FIG. 2. Primary leaf of a bean plant which was inoculated at the cotyledon node.

On the pods. The lesions on the pods are not unlike those caused by *Phyt. phaseoli* (Pl. LV, A). The greatest difference is that they seem to spread more rapidly in the field and are probably more water-soaked in appearance. When they dry out it is very difficult to distinguish between

the two. Individual infections are more or less round, but they anastomose and upon drying become irregular in shape, and brick red to brown. The spots caused by *Phyt. phaseoli* frequently have a slight, yellowish incrustation covering them, while those due to the white organism have a silvery incrustation. It should be stated, however, that the incrustation in both diseases occurs on the larger and more severe lesions and then are not long lived. They are easily brushed off. Consequently this character can seldom be used to distinguish between the two diseases.

On the seed. Very small spots may occur on the seeds, or the entire seed may be practically destroyed. On colored seed it is impossible to distinguish the new disease from the other bacterial diseases of the bean or from the small spots due to the fungous pathogene, *Colletotrichum lindemuthianum* (Sacc. et Magn.) B. et C. On white seeds the distinguishing characteristic is the maize yellow to cream color[4] of affected areas. Seeds severely infected are small and wrinkled and may be completely colored by the pathogene.

The bacteria may enter the seed either through the pod or through the vascular system. The pathogene undoubtedly overwinters on the seed as do the causal organisms of the other two bacterial diseases of the bean.

ISOLATION

The organism causing this disease has been isolated a number of times from specimens collected in various parts of New York State. It has been obtained from infected seeds, from pod spots, from lesions on the leaves, and from the interior of stems of systemically infected plants. Where there are visible necrotic symptoms of the disease on the plant, the bacteria have always been in abundance. Maceration of small portions of lesions in sterilized water and the use of loops full of this water in dilution plates has always yielded the organism.

In studying the pathogene the writer used three cultures isolated from various places in New York State. Two came from Wyoming County, one isolated from a Perry Marrow plant and one from a Wells' Red Kidney plant. The third culture was isolated from a Red Kidney plant collected in Wayne County. No variation was observed in the three.

MORPHOLOGY

The pathogene is a small rod with rounded ends, occurring singly or in pairs. On beef-extract agar which is somewhat acid long filaments are formed (Pl. LV, C). The organism stains readily with Ziehl's carbol

[4] Ridgeway, R. Color standards and color nomenclature. 44 pp., 53 col. pl. Washington, D. C. 1912.

fuchsin, gentian violet, and Loeffler's methylene blue. With all stains the contents of the organism appear to be granular, and very frequently a definite bipolar staining is obtained. From a 24-hour culture on beef-extract agar (pH 6.9) the rods stained with carbol fuchsin measure 1 to 3 μ by 0.5 to 1.25 μ.

The organism is actively motile, and on beef-extract agar one single polar flagellum has been demonstrated. Capsules have not been found.

CULTURAL CHARACTERISTICS

All cultures were kept in an incubator at 27° C. unless otherwise noted.

Beef-extract agar stroke (pH 7). Growth is scanty to moderate appearing in about 24 hours, colorless to white, at times creamy, filiform, with smooth, glistening surface, translucent. The consistency is at first butyrous, but becomes brittle with age. The medium remains unchanged. No odor detected.

Poured agar plates. The surface colonies develop at a moderate rate, becoming circular and glistening with a zonation about the edge. They are somewhat colorless to white and translucent. Their peculiarities may be observed in Plate LV, D.

Beef extract gelatin stab (pH 7). Kept at 20°–21° C. for six weeks. No liquefaction. A good white surface growth was formed, and a feathery, arborescent growth along line of puncture.

Poured gelatin plates. Colonies are slow growing, circular, raised, and slightly wrinkled. They are not very characteristic.

Beef extract bouillon (pH 7.1). In beef extract bouillon the optimum growth is reached at the end of 24 hours. The medium becomes cloudy, and a delicate pellicle is formed which breaks up if disturbed. A light precipitate is produced.

Litmus milk. A bluish ring appears at the surface of the milk in two to three days, and the color gradually extends throughout the medium. At the end of two weeks there appears to be a slight reduction of the litmus at the bottom of the tube. This whitens somewhat and then turns muddy, the top half remaining blue. At the end of six weeks no further change takes place.

Potato cylinders. A very scant growth barely visible to the eye is produced. Starch is not digested, but the medium is somewhat darkened.

Starch agar plates. When tested with iodin solution at the end of 7 and 14 days no digestion was observed.

Uschinsky's solution. A white turbid growth at first, soon becoming yellow green. Color appears first in a layer at the surface of the medium and gradually extends to the bottom. Pellicle formed which breaks up, leaving a heavy sediment.

Fermi's solution. In 24 hours, growth becomes visible. The medium later becomes cloudy and very slightly green in color. A heavy pellicle forms, which, upon being disturbed, falls to the bottom.

Cohn's solution. No visible growth.

Dextrose broth.[5] In fermentation tubes the medium becomes slightly cloudy in 24 hours. A heavy pellicle is then formed; the growth in the open arm becomes very cloudy and gradually extends into the closed arm. Acid is noted on the second day but a reaction shortly sets in and the medium becomes alkaline. No gas is produced.

Saccharose broth. In the open arm of a fermentation tube growth begins in about 24 hours. The medium is slightly cloudy at first with a light pellicle, progressing until it is turbid with a heavy pellicle. A good cloudy condition extends throughout into the closed arm. On the second day a slight acidity may be detected, but the medium shortly becomes alkaline again. No gas is produced.

Lactose broth. Growth begins in approximately 24 hours, and the medium becomes cloudy with a light pellicle. There is no clouding of the closed arm, and no acid or gas is produced.

Maltose broth. A cloudy growth appears in the bowl at the end of 24 hours. Later a light pellicle is formed. Acid or gas is not produced.

Nitrate reduction. Nitrates are not reduced. The organism was grown in a nitrate bouillon and test made at the end of 4 and 10 days with sulphanilic acid and alpha naphthalamine in 5N acetic acid.

Hydrogen sulphid production. Stabs were made in a lead acetate agar but no blackening or browning of the medium occurred along the line of inoculation. Positive results were obtained on this agar with *Bact. coli.*

Indol production. Indol was not produced. The medium used was Dunham's solution which had been prepared with a pre-war Witte peptone. The Ehrlich test was employed.

Oxygen relations. The organism grew well in the closed arm of fermentation tubes in both dextrose and saccharose broth. In shake agar cultures of ordinary beef extract agar, colonies were formed 3.5 cm. below the surface.

Longevity. The organism has lived for 12 months on beef extract agar kept at a temperature of 3°–6° C. Its virulence has not been impaired.

Thermal death point tests. Test tubes of a uniform thickness and containing 10 cc. of a beef-extract broth were used in these experiments. Two

[5] The sugar broths used here were the dehydrated broths obtained from the Digestive Ferments Co. They were the ordinary beef-extract broth to which the sugar to be tested and Andrade indicator had been added. After sterilization the reaction varied with the lot of media prepared from pH 7.3 to pH 7.6.

lots of broth were used at various times having a pH value of 6.9 and 7.0. To these tubes were transferred two large loops full of a 24-hour broth culture, and the tubes were immersed in a water bath for 10 minutes. The water was kept agitated and accurate temperature readings were made continuously with a certified thermometer. From a number of such experiments it was determined that the thermal death point was approximately 49° C.

NOMENCLATURE

If the above descriptions of the morphology and cultural characters of this organism are examined, it will be noted that they agree remarkably well with the descriptions of *Phyt. medicaginis* Sackett.[6] Other closely related organisms are *Phyt. sojae* Wolf, *Phyt. glycineum* Coerper and *Phyt. trifoliorum* Jones *et al.* Certain minor differences, however, may be found between the alfalfa organism and the bean organism, notably in the reaction of the pathogenes on several of the various media used. These are recorded in table 1. It must be admitted that these differences are slight and are ones which should scarcely be used as specific. In the first few cases they

TABLE 1.—*Differences noted between Phyt. medicaginis and the bean pathogene, Phyt. medicaginis var. phaseolicola*

Phyt. medicaginis	*Phyt. medicaginis* var. *phaseolicola*
Flagella 1–4, bipolar	Flagellum 1, polar
Nutrient agar becomes fluorescent	No change noted
No acid produced in dextrose broth	Slight acid produced in dextrose broth
No acid produced in saccharose broth	Slight acid produced in saccharose broth
No growth in closed arm of fermentation tubes in the above broths	Growth in closed arm of fermentation tubes in the above broths
Pathogenic to alfalfa	No infection obtained on alfalfa

even might be attributed to differences in technique or interpretation. When the matter was placed before Dr. W. G. Sackett, he was inclined to consider this organism a variety of *Phyt. medicaginis*. It should be stated here, however, that there was no culture of the alfalfa organism obtainable for comparison. The differences listed in table 1, especially the difference in pathogenicity, nevertheless appear sufficiently great to be considered varietal. For these reasons the bean organism is treated as a variety, and the name *phaseolicola* is given to it.

[6] Sackett, Walter G. A bacterial disease of alfalfa. Col. Agr. Exp. Sta. Bul. 158: 1–32. 1910.

TECHNICAL DESCRIPTION

Phytomonas medicaginis var. **phaseolicola** n. var.

Non-spore-forming rods with rounded ends; occurring singly or in pairs; cells 1.5 to 3 μ by 0.5 to 1.25 μ; on some media forming long filaments; motile by means of one polar flagellum; Gram negative, and not acid fast; when stained with carbol fuchsin or methylene blue showing more deeply stained areas which frequently are bipolar; a facultative anaerobe.

Colonies on nutrient agar, white to colorless, or at times, creamy, slow to moderate growing, concentrically ringed with edges undulate; gelatin colonies, slow growing, circular, raised, and somewhat wrinkled; agar streak, a moderate growth, colorless to white, flat, glistening, translucent, butyrous but later brittle; in nutrient broth a cloudy growth within 24 hours and pellicle formed later; litmus milk becomes an intense blue, but no curd formation nor peptonization occurs; produces a small amount of acid in dextrose and saccharose, but reverts almost immediately to the alkaline side; no acid in lactose or maltose; no gas formed in the above sugars; produces neither nitrite nor gas in nitrate media; does not liquefy gelatin, nor digest starch; no production of indol in Dunham's solution; no hydrogen sulfide produced in basic lead acetate agar; in Uschinsky's solution, a light yellow green turbid growth; a slight trace of green in Fermi's solution; no growth in Cohn's solution; thermal death point approximately 49° C.

Pathogenic on the common bean (*Phaseolus vulgaris* L.), the lima bean (*P. lunatus* L.), and the scarlet runner bean (*P. multiflorus* L.).

INOCULATION EXPERIMENTS

In conducting inoculation experiments several procedures were used, the most usual of which was the introduction of the pathogene into the stem by pricking with a needle or sharp-pointed scalpel carrying the bacteria. These wounds were inflicted either at the cotyledon or first leaf node. When conducting these experiments in the greenhouse no further care of the plants was necessary; that is, they were not placed under excessively moist conditions for the pathogene to establish itself. Several hundreds of such experiments were conducted during the last year and a half, and in every case excellent infection was obtained, except where varieties more or less resistant were used.

When Red Kidney seedlings were taken as the host plant and the experiments were conducted in a greenhouse held at approximately 80° F., the incubation period was about four days. In some of the cooler houses a week or so was required for the first symptoms of the disease to appear, and, if more mature plants were used, the time was still longer. Good infection,

however, occurred at 65° F., while infection at this temperature with *Phyt. phaseoli* was exceedingly light, and but moderate with *Phyt. flaccumfaciens.*

When bean pods were inoculated by pricking with a needle bearing the pathogene, distinct water-soaked areas about the point of puncture could be detected readily within three days. On young pods these lesions spread rapidly and not infrequently involved considerable tissue. A high temperature was maintained throughout these experiments.

That the pathogene requires a wound through which to enter the plant seems to be indicated by the following experiments. In one group of experiments 12 Red Kidney seedlings were used, and in another group 12 Red Kidney seedlings and 5 Robust pea bean seedlings. These were sprayed with a water suspension of the pathogene and held under moist conditions over night. In each case several Red Kidney seedlings were inoculated by pricking them at the cotyledon node with needles which had been smeared with a culture of the pathogene. These latter became infected, but in no case did one of the sprayed plants show symptoms of the disease. At another time six Red Kidney pods were painted with a water suspension of the bacteria and two of them pricked with a needle. The disease quickly appeared on the wounded pods, but the uninjured pods remained healthy. Further experiments, however, should be conducted before coming to any definite conclusions on this point.

VARIETAL SUSCEPTIBILITY

While no extensive experiments have been conducted to determine the varietal susceptibility of beans to this disease, yet certain observations have been made which should be reported. The Red Kidney, including the Wells' strain, is without doubt the most susceptible. Whole fields of this variety have been destroyed; and when seedlings are artificially inoculated in the greenhouse, over 75 per cent of them die. The White Kidney, too, is probably equally susceptible. The White Marrow and the Pea bean are somewhat more resistant, although they should never be recommended as resistant varieties. Inoculations in the greenhouse do not, as a rule, completely kill them.

The results of the following experiments indicate that practical resistance may be found for this disease. Nine seedlings of the White Kidney variety, 15 of Scotia, 16 of Refugee or Thousand-to-one, 6 of the Dwarf Grenell Rust Proof Wax, and 14 of London Horticultural were inoculated by pricking at the first leaf node. At the end of a week all showed unmistakable symptoms of the disease with the exception of Refugee and Scotia. One or two plants of these latter varieties finally showed faint symptoms, but all the plants grew to maturity and produced very satisfactory yields.

CROSS INOCULATIONS

A great many inoculation experiments were conducted with *Phyt. medicaginis* var. *phaseolicola* on a number of species of plants, mostly legumes, to determine, if possible, other hosts for the pathogene. To be sure that in all experiments a virulent culture of the organism was being used, Red

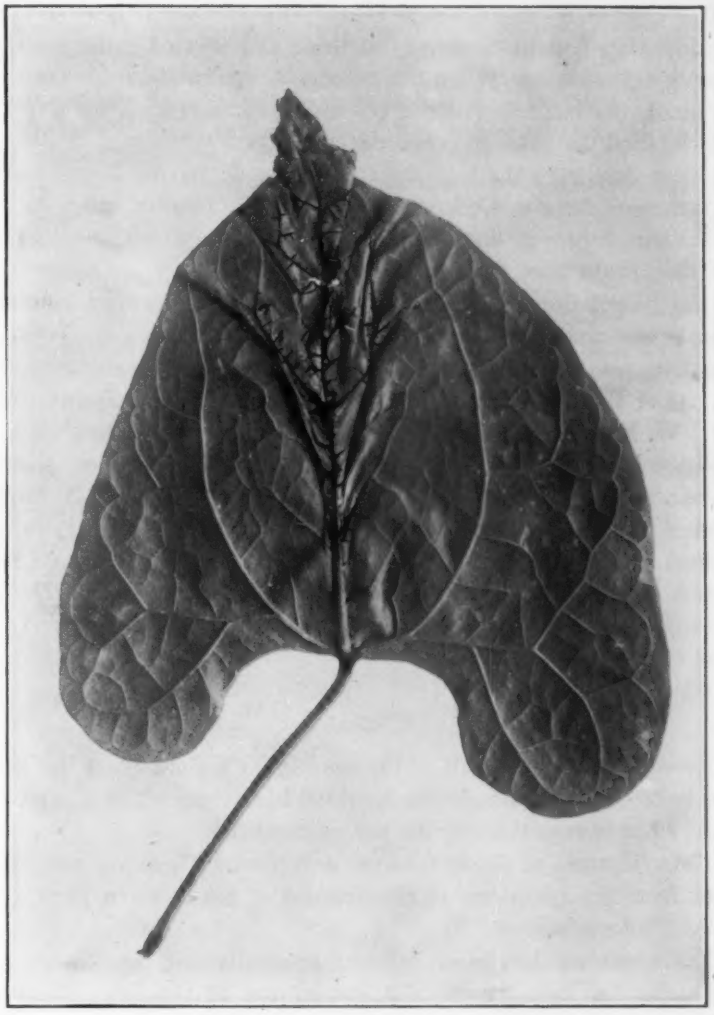


FIG. 3. Leaf of a lima bean plant showing killing of the veins due to *Phyt. medicaginis* var. *phaseolicola* infecting the vascular system. The plant was inoculated artificially at a lower node on the stem.

Kidney bean seedlings were inoculated and held under conditions similar to those under which the other plants were being tested. Without exception these check plants became infected. However, only two other species were found susceptible to this pathogene: these were of the genus *Phaseolus*—the lima bean (*P. lunatus* L.), and the scarlet runner bean (*P. multiflorus* Willd.).

On the lima bean the organism is quite virulent, and the disease produced is similar to that on the common bean. One symptom, however, in artificially inoculated plants was very striking and seemed to be more or less peculiar to this species. When the pathogene was introduced into the stem of the plant, the bacteria entered the leaves through the vascular bundles and there killed the veins in certain areas. These veins turned a very dark red to black and gave the leaf the appearance of having been infected by the anthracnose fungus, *Colletotrichum lindemuthianum* (Fig. 3). These diseased areas, however, flag and wilt very rapidly, which is not characteristic of the anthracnose disease.

In the inoculation experiments conducted on the scarlet runner bean the disease was much less severe.

Negative results were obtained with a number of legumes and other plants. The list is as follows: The adzuki bean (*Phaseolus angularis* (Willd.) W. F. Wight), the mung bean (*Phaseolus aureus* Roxb.), the cowpea (*Vigna sinensis* (L.) Endl.), the soy bean (*Glycine max* Merr.), the broad bean (*Vicia faba* L.), the garden pea (*Pisum sativum* L.), the flowering lupine (*Lupinus polyphyllus* Lindl. var. *albiflorus* Hort.), red clover (*Trifolium pratense* L.), alfalfa (*Medicago sativa* L.), potato (*Solanum tuberosum* L.), cotton (*Gossypium hirsutum* L.), sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.), and tobacco (*Nicotiana tabacum* L.).

SUMMARY

1. A severe blight and wilt of the bean in New York State has been discovered to be due to a hitherto undescribed bacterium which has been given the name *Phytomonas medicaginis* var. *phaseolicola*.

2. The symptoms of the disease are described. These are not strikingly different from the symptoms of the diseases of bean due to *Phyt. phaseoli* and *Phyt. flaccumfaciens*.

3. The organism has been isolated from diseased specimens and its pathogenicity has been proved. A description of the morphology of the pathogene together with its cultural characters is given.

4. In addition to the common bean (*Phaseolus vulgaris* L.), the scarlet runner bean (*P. multiflorus*) and the lima bean (*P. lunatus* L.) have proved susceptible.

CORNELL UNIVERSITY,
ITHACA, N. Y.

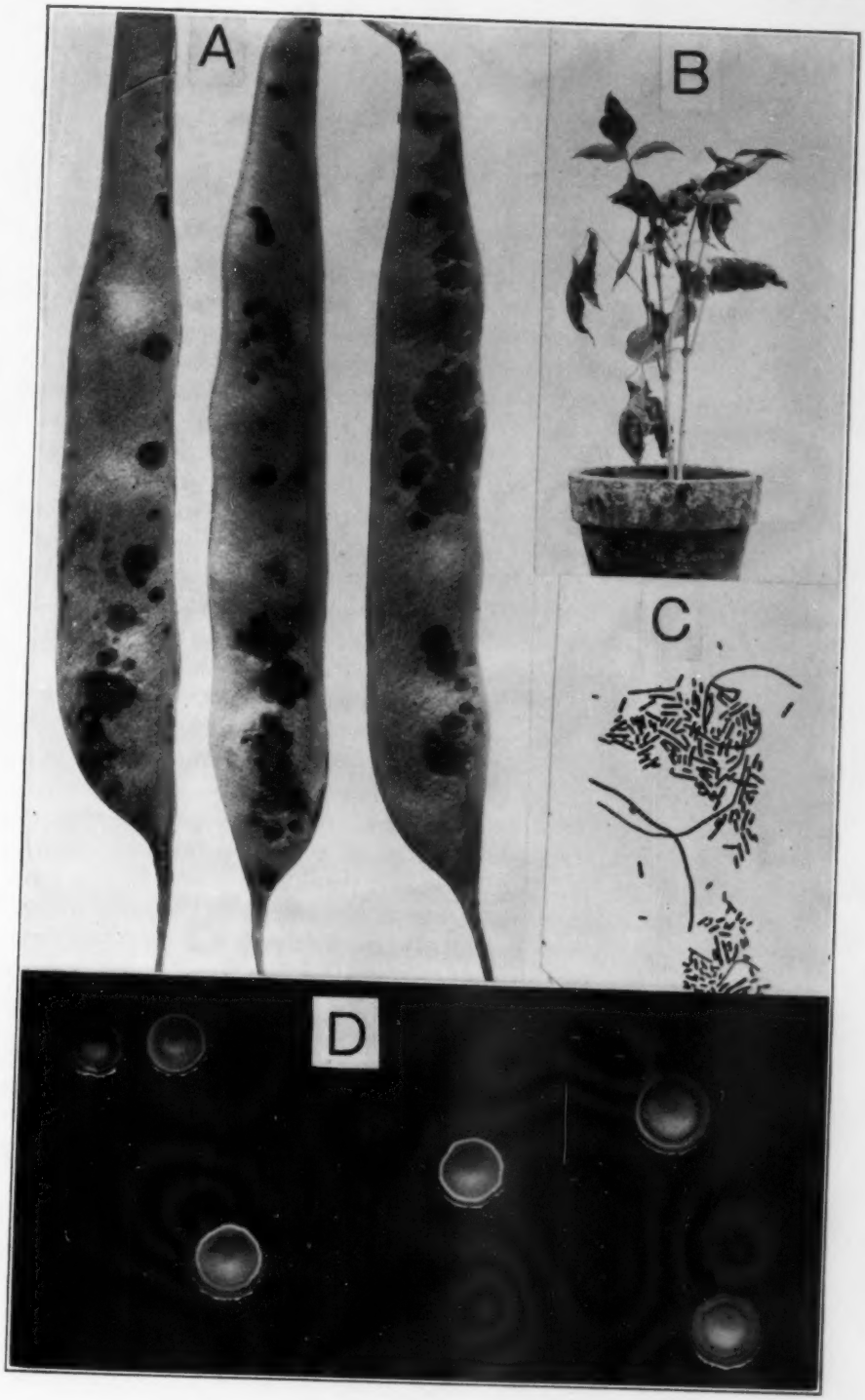
EXPLANATION OF PLATE LV

A. Pods of the Red Kidney bean showing water-soaked lesions from which *Phyt. medicaginis* var. *phaseolicola* was isolated.

B. Wilt of two Wells' Red Kidney plants due to artificial inoculation of the plants at the first leaf node with *Phyt. medicaginis* var. *phaseolicola*.

C. Photomicrograph of the bacteria from acid beef-extract agar showing filaments. Organisms stained with carbol fuchsin.

D. Colonies of *Phyt. medicaginis* var. *phaseolicola* on beef-extract agar 14 days old. 2 ×.


A
B
C
D

NET NECROSIS OF POTATO

D. ATANASOFF

GENERAL REMARKS

Net necrosis of potato was first described by W. A. Orton (10) in 1914 as follows: "It is characterized by the occurrence of narrow streaks or dots of browned tissues outside of the vascular ring and extending from the stem end into the tuber for a considerable distance or entirely through it. These brown tissues are free from fungi or bacteria." Orton was not sure of the cause of net necrosis.

The above short but exact description of the disease, to which Orton gave the name net necrosis, together with his illustrations, is sufficient to enable us to recognize with certainty a little understood, but not uncommon, potato disease.

C. R. Orton (9), in a short note in 1916, described the same disease. His illustration of the disease resembles the disease discussed in this paper.

Güssow (6), also in 1916, writes of this disease as follows: "During this year a considerable number of potatoes were found on being cut to have brownish internal discolorations which at first are arranged in the form of a ring, but later on become quite irregular in their distribution. These discolorations correspond to the vascular bundles . . ." Güssow observed that the disease begins at the stem end and gradually travels along the vascular bundles towards the eye-end, turning the tissues brown as it proceeds. He did not find any bacteria or fungi associated with the disease. The disease is distributed over a wide area, having been observed in New Brunswick and British Columbia. In planting tubers showing net necrosis, Güssow was surprised to find that in almost every case the disease was hereditary, *i.e.*, it was transmitted to the new tubers.

Coons (3) writes of net necrosis: "The blackened flesh is free from parasitic organisms and the disease is believed to be of some such nature as mosaic or leaf roll." This is the first mention that net necrosis is a virus disease, although Güssow's experiments suggest this. Cook (2) and Jones *et al.* (7) also state that net necrosis is inheritable from generation to generation.

Schultz and Folsom (13, 14, 15) were the first to make a study of net necrosis. They write (13) that "in Maine net necrosis has been consistently accompanied by leafroll. It may be that net necrosis, a tuber phloem-necrosis, is caused by the same virus as is leafroll or phloem-necrosis." This

hypothesis, they write, explains the available facts and explains them at least as well as does the assumption that the two diseases are caused by different viruses. According to them "net necrosis is apparently a leafroll symptom, being a discoloration which results from tuber phloem-necrosis and which appears more often as conditions of variety, receney of infection and weight of tuber are more favorable. It develops in the dormant tubers without relation to differences in the storage temperature. When it occurs as a symptom of leafroll, the effects of the latter are still more detrimental, one being a decided spindliness of the sprouts. Transmission and control of leafroll are thus concerned indirectly with net necrosis and spindling-sprout."

Gilbert (5), two years later, also came to the conclusion that "Net necrosis . . . is definitely and closely correlated with spindling-sprout of the tuber and with leafroll of the plant." This has become meanwhile the prevailing opinion among plant pathologists.

Murphy and McKay (8), on the other hand, write: "Much emphasis has been laid in America on the relationship of leaf-roll to net necrosis, that is, the presence of fine net-like strands of dead tissues in the neighborhood of the vascular ring. This feature was not found to be by any means invariably associated with leafroll."

Anderson and Millar (1), in an anonymous paper, write also: "American observers state that the tubers of plants affected with leafroll frequently show net necrosis—the presence of fine net-like strands of dead tissue in the outer part of the flesh of the tuber—associated with a spindling condition of the sprouts, but these features have not been found in Scotland to be symptomatic of leafroll."

The evidence gathered by the writer during the last three years regarding the nature of net necrosis is entirely contradictory to the results obtained by Schultz and Folsom and later also by Gilbert. It shows conclusively that net necrosis, as described and illustrated by W. A. Orton, is not a tuber symptom of leafroll, but that it is a different disease, which attacks the potato plant independently of leafroll. It is not a disease of the tuber alone but manifests itself in various ways also on the leaves of the affected plants. The leaf symptoms of this disease vary, according to variety, from what has been described by Quanjer (11, 12) as Aucuba mosaic to complete absence of Aucuba mosaic or development of Aucuba mosaic symptoms only at the beginning or at the end of the growing period.

DESCRIPTION OF THE DISEASE

Plants infected with net necrosis grow normally, are vigorous, attain normal size and show no morphologic changes whatever. Their yield is good. No apparent running out of the infected plants takes place even as far as the fourth generation after infection.

The symptoms of net necrosis vary considerably according to the variety. Some of the symptoms may be entirely absent in some varieties and very pronounced in others.

Leaf symptoms. In some varieties the leaf symptoms of net necrosis form what has been described by Quanjer (11, 12) and later by others as Aucuba mosaic of the potato plant. The affected plants and their leaves are as normal and as vigorous as healthy plants of the same variety. The leaves of such plants, however, are sprinkled or spotted with more or less rounded, bright yellow spots (Fig. 5). These spots are of considerable size, as large as one centimeter in diameter in some varieties (Midlothian Early) and very small in others. Often they coalesce, forming large, irregular,

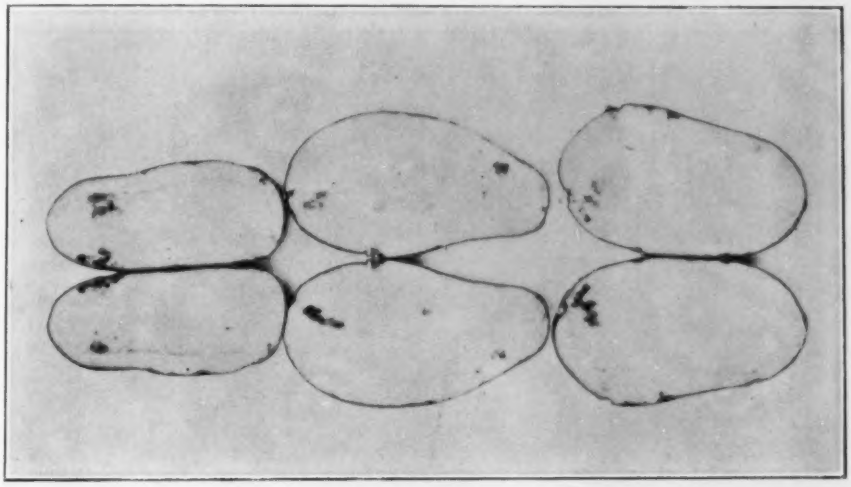


FIG. 1. Ashleaf tubers showing slight net necrosis. Rusty spots scattered irregularly all over the tubers.

yellow blotches on the leaves. When some of the older, fleshy leaves are heavily spotted, they preserve their normal shape but become blistered, *i.e.*, the yellow areas become slightly elevated above the normal green portions of the leaf. In the varieties Schotsche Muis (Midlothian Early, Duke of York), Koksiaan (May Queen), Paul Krüger (President), Ninetyfold, etc., the leaves can be so severely spotted as to resemble closely the variegation of *Aucuba japonica*—hence Quanjer's name Aucuba mosaic for this disease. In other varieties the yellow spotting of the leaves is much less pronounced and may be limited to only a few of the leaves of a plant. Such are the varieties Immune Ashleaf, Eigenheimer, Industrie, Bintje, etc. But in these varieties, also, the yellow spotting becomes more pronounced towards the end of the growing period. Other varieties, as Roode Star for instance,

show very slight yellow spotting and blistering of the lower leaves. In this case the yellow spotting resembles superficially slight frost injury. The yellow spotting of this variety, however, soon disappears or becomes less pronounced. On some of the affected leaves the yellow spots become brown and necrotic, and the browning soon extends over the top and edges of the leaf, advancing downwards until the whole leaf dies and falls off prematurely. Still other varieties, as Success, do not show any yellow spotting whatever in any stage of their development. In such cases the young plants of affected mother tubers come above the ground quite normal and absolutely free from any disease symptoms. Before the plants reach any size, however, there appear on the tips and edges of their lower leaves irregular brown to black spots as in the case of mature secondary leafroll plants. The brown

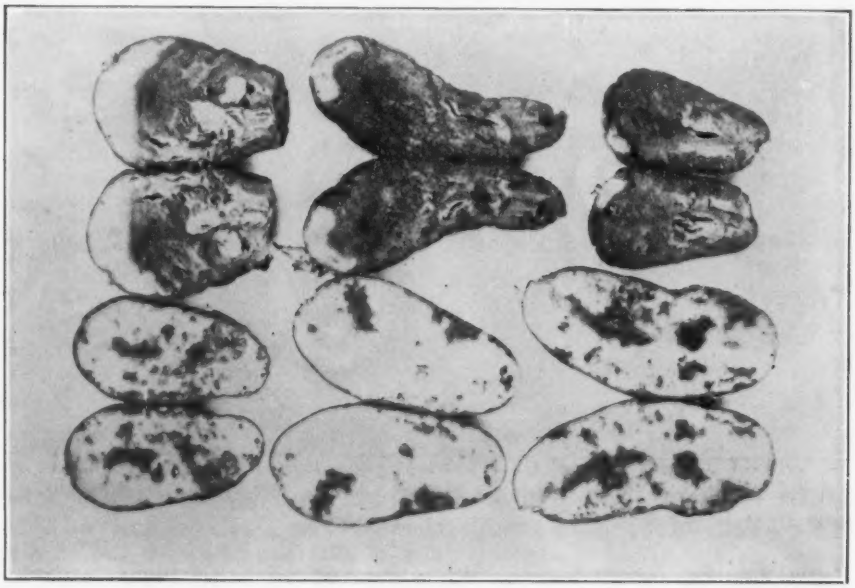


FIG. 2. Ashleaf tubers showing severe symptoms of net necrosis. Note that some tubers are wholly destroyed and are being invaded by fungi.

spots grow in area, coalesce and gradually extend over the whole upper portion of the leaves. These affected leaves thus shrivel, die, and fall off prematurely, disclosing the base of the stems. The uppermost leaves of such plants remain permanently free from any pathogenic changes. Finally there are varieties, like Bravo for instance, on which leaf symptoms have never been seen.

Of the numerous plants belonging to a large number of different varieties artificially and naturally infected with net necrosis, none ever showed any

leafroll symptoms, although all varieties used are known to be susceptible to and capable of developing pronounced symptoms of leafroll when infected with this disease.

Tuber symptoms. The tuber symptoms of net necrosis also vary according to variety. They are most pronounced and develop most rapidly in the early varieties, Schotsche Muis (Midlothian Early) and Ashleaf (Fig. 2). In most varieties the tuber symptoms are absent or only slightly developed at the time of digging. It is in the storage houses and during the winter that the tuber symptoms of net necrosis, if ever, become apparent and reach maximum development. The early varieties, however, show distinct net necrosis of the tubers at the time of lifting, which may be as early as the beginning of June. In these varieties the necrosis of the tubers increases with great rapidity, and within two months after lifting the entire sub-

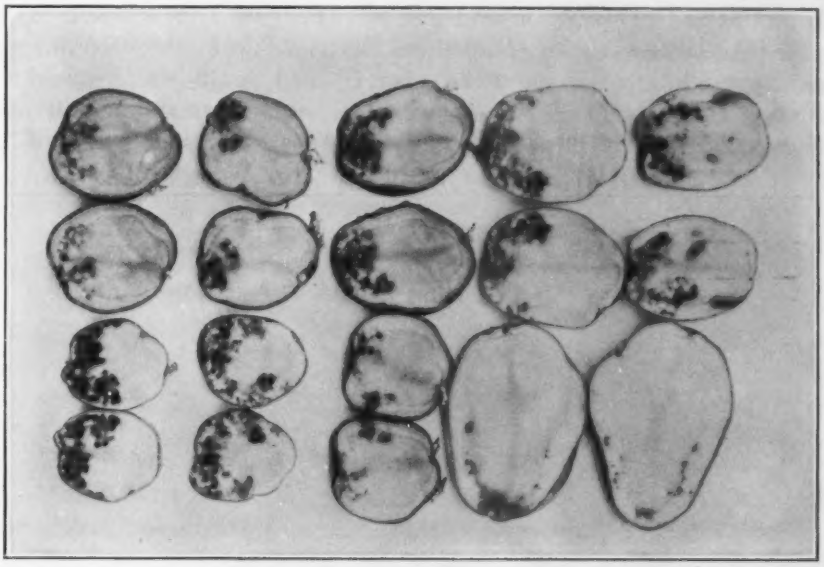


FIG. 3. Roode Star tubers showing net necrosis. Note that the brown spots first appear at the rhizome-end and advance gradually towards the eye-end of the tuber.

stance of some tubers may become wholly brown and necrotic (Fig. 2). Long before this stage the tubers have become unfit for consumption. In the varieties Midlothian Early and Ashleaf the tuber necrosis may develop very rapidly and destroy completely some of the infected tubers, while other tubers originating from the same plant may show only slight necrosis, which does not develop further. Such tubers remain alive until spring and serve for further propagation of the disease. Freshly dug tubers of the above

two varieties, when fully ripe, will show, upon careful examination, more or less pronounced rusty brown spots deep under the periderm in the flesh of the tuber. Gradually these rusty spots extend upwards against and along the periderm, forming large rusty brown, watery blotches under the periderm which later become dry. Soon the periderm dries out and begins to shrivel or sink in over the necrotic area. This condition of the diseased tuber, as well as the other symptoms, resembles very much the symptoms of Phytophthora tuber infections, and there can be no question but that the tubers infected with net necrosis have often been considered as being infected with *Phytophthora*.

Potato tubers affected with net necrosis, when cut open longitudinally, show very pronounced rusty brown blotches, spots, or streaks in the parenchyma. In some varieties (Roode Star) the first rusty spots appear in the parenchyma tissues immediately under the rhizome scar of the tuber (Fig. 3) and extend gradually inward and upward toward the eye end of the tuber. In other varieties (Midlothian Early, Ashleaf) the necrotic spots may appear in any portion of the tuber (Figs. 1 and 2), but very seldom or never at the stem end. In both cases the rusty spots appear outside of the vascular ring in the parenchyma of the tuber, as first stated by W. A.

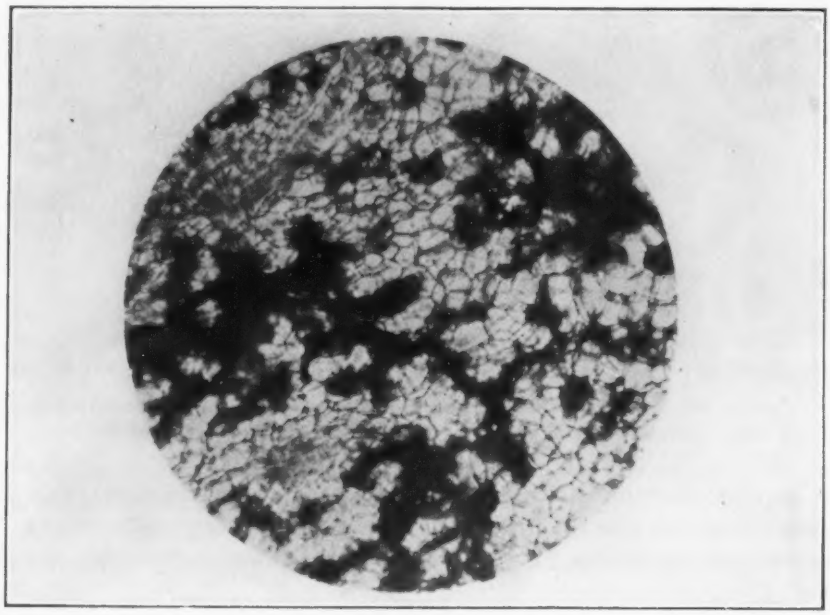


FIG. 4. Photomicrograph of a section of potato tuber of the variety Roode Star infected with net necrosis.

Orton (10), and have no relation to the phloem, as claimed by Schultz and Folsom (13). This difference in symptoms of net necrosis, as studied by Schultz and Folsom on the one hand and by Orton and the writer on the other hand, may be due to the fact that different varieties react differently to the disease. It also may be due to the fact that Schulz and Folsom's net necrosis material was infected also with leafroll. The presence of the latter in the tubers, as is known, may lead to the development of phloem necrosis in some cases. The probability that Schultz and Folsom have worked with an entirely different pathologic condition of the potato tuber than the one observed by Orton and the one discussed in this paper is very small. The illustration of net necrosis shown in Plate 2B of the paper by Schultz and Folsom (13) agrees completely with the net necrosis described and illustrated by Orton (10) and illustrated in this paper by the variety Roode Star.

Microscopic examination of the tubers before the appearance of macroscopic symptoms of the disease shows that the cell contents of individual cells or small groups of cells in the tuber parenchyma are brown colored and disorganized. The browning extends gradually to the neighboring cells and becomes visible to the unaided eye. The number of spots increases, and some of them coalesce, forming a network (Fig. 4). Such tubers, when planted, give rise to large and vigorous plants.

EXPERIMENTAL RESULTS

During the spring of 1922, after the appearance of Schultz and Folsom's paper (13) in which they claimed that net necrosis is a tuber symptom of leafroll, many hundreds of tubers from leafroll plants belonging to a large number of different varieties were cut and examined under the direction of Professor Quanjer, but in no case was net necrosis to be seen. At the same time there were at the writer's disposal several bushels of tubers of the variety Success and several hundred tubers of the variety Ashleaf originating from nonleafroll plants, which had almost 100 per cent net necrosis.

The disease present in this material closely resembled the Phytophthora tuber infection. A considerable number of the tubers were cut open and left in a moist chamber for several days, but in no case did any *Phytophthora* develop on them, as is usually the case with tubers infected with this fungus. A number of the infected tubers of both varieties were planted to see whether they would show any leafroll symptoms. In all cases the tubers gave rise to vigorous and leafroll-free plants. Since 1922 a portion of the Ashleaf material has been planted every year by the writer, and in all cases the plants have been free from leafroll; but in all seasons they showed more or less pronounced Aucuba mosaic on the leaves and net necrosis in the tubers.

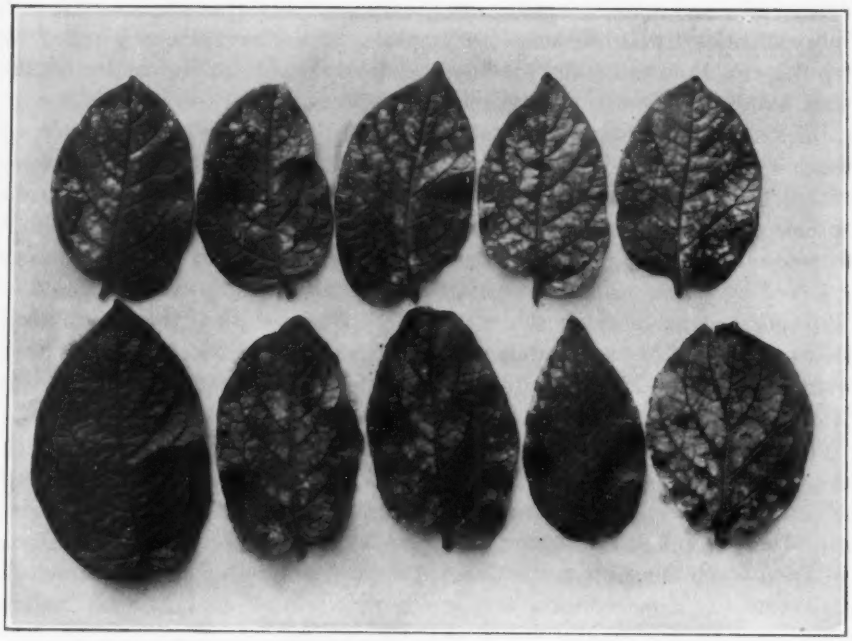


FIG. 5. Potato leaflets of the variety Midlothian Early showing Aucuba mosaic.

Early in 1924 Dorst (4) published his work on bud variation in the potato plant, in which he reported that a selection of the variety Roode Star, contrary to the general experience, developed internal brown spot on all soils. Dorst gave several tubers of this selection to Prof. Quanjer, who turned them over to the writer for further investigation. Examination showed that it was not affected with internal brown spot, but with net necrosis. When a portion of this material was grafted to healthy tubers of Midlothian Early, the necrotic condition was transmitted to the grafted healthy tubers. The rest of the material was planted on heavy clay soil, on which internal brown spot has never been seen in Holland. The resulting plants grew normally, showed no leafroll, but slight temporary symptoms of Aucuba mosaic. Their tubers developed net necrosis after lifting in all cases. While the above plants were still young and growing vigorously, their shoots were grafted on young Midlothian Early plants. The grafted plants were kept isolated in the hothouses and were fumigated regularly to keep them free from aphids. These plants did not develop distinct Aucuba mosaic symptoms. Their tubers, however, showed very severe net necrosis, while those of the check plants were free from net necrosis. The absence of Aucuba mosaic symptoms on the grafted plants is due to the fact

that the plants were not growing normally in the hothouse. Net necrosis tubers planted during the spring of 1925 in the hothouse also failed to show Aucuba mosaic in a distinct form, although the same material planted outside gave rise to plants which showed distinct Aucuba mosaic.

In the fall of 1924 the writer received from Dr. Oortwijn Botjes several hundreds of disease-free tubers of the variety Roode Star. Upon examination all tubers without exception were found to be affected with net necrosis. Dr. Oortwijn Botjes had also observed that the tubers of this lot had rusty brown spots at the rhizome end, but he, like many others, considered this due to *Phytophthora*. Although all this material was infected with net necrosis, it originated from plants that had been grown isolated from other plants for several years and which had never shown any symptoms of any of the degeneration diseases. This material, therefore, seemed especially suited for infection experiments, because it could be taken for granted, according to experience with the disease-free potato seed furnished by Oortwijn Botjes, that it was free from other virus diseases as leafroll, mosaic, etc.

The first step in the study of this, as well as of the other, net necrosis material was to establish whether the tubers showing net necrosis, give rise to leafroll plants when planted. Twenty tubers of the Roode Star material showing net necrosis were planted on light sandy soil. The resulting plants were normal and vigorous, and none of them showed any leafroll symptoms whatever. Several hundred tubers of the same lot were planted in a second field on heavy clay soil. They also gave rise to normal, and absolutely leafroll-free plants.

Ten tubers of the same material, showing net necrosis, were grafted early in February, 1925, with tubers of the variety Paul Krüger (President), originating from leafroll plants. The grafted tubers were incubated for two months at room temperature. The Paul Krüger tuber portions were then removed and the net necrosis portions planted out of doors. Seven of the ten tubers thus treated developed, within a short time, typical and very pronounced symptoms of secondary leafroll. This experiment shows conclusively that net necrosis is not a tuber symptom of leafroll.

In order to see the effect of net necrosis on different potato varieties, ten disease-free tubers of each of the varieties Bravo, Midlothian Early, Eigenheimer, Immune Ashleaf, Bintje, May Queen, Industrie, and Success were grafted early in February with Roode Star tubers affected with net necrosis. The grafted tubers were kept at room temperature for about 50 days and were planted March 27 on sandy soil well fertilized with artificial fertilizers. Ten tubers of the naturally infected Roode Star from Oortwijn Botjes were also planted with the above varieties. All tubers gave rise to normal and vigorous plants. All Roode Star plants from the naturally infected tubers showed slight Aucuba mosaic symptoms while still young. However,

gradually all Aucuba mosaic symptoms disappeared completely. Meanwhile some of the lower leaves became rusty and died. This, however, does not attract much attention and is never very distinct in this variety. Towards the end of the growing period the same plants were normal, large, and vigorous. Such plants would be considered as perfectly healthy. This explains why Dr. Oortwijn Botjes's whole stock of this variety was 100 per cent net necrosis.

The variety Bravo in this experiment showed no leaf symptoms. All ten plants were normal and free from any disease symptoms.

The variety Midlothian Early (Schotsche Muis), on the other hand, developed in all ten plants very pronounced Aucuba mosaic on the leaves, which became more and more pronounced during and towards the end of the growing period. At this time most of the riper tubers showed distinct necrosis as described for this variety. The rest of the tubers in all cases later developed net necrosis. The check plants of this variety, more than 50 in number, did not show any Aucuba mosaic, and only four per cent of their tubers developed net necrosis in storage.

All ten plants of the variety Eigenheimer showed Aucuba mosaic, but the symptoms were less pronounced and usually limited to a few leaves of each plant. Here also the plants grew normally and were very vigorous. The check plants of this variety were also free from Aucuba mosaic.

The ten Immune Ashleaf plants also showed distinct Aucuba mosaic, slight at first but later pronounced. Here, however, the yellow spots were not bright yellow, as in Midlothian Early, but more of a yellowish green tint. The check plants did not show any Aucuba mosaic.

All ten plants of the variety Bintje showed Aucuba mosaic. The yellow spotting, although quite distinct, was not pronounced at first, but became more and more so toward the end of the growing period. Here also all check plants remained healthy.

The ten May Queen (Koksiaan) plants behaved as the Bintje plants. The check plants remained free from Aucuba mosaic.

The Industrie plants at an early stage showed distinct Aucuba mosaic symptoms, consisting of numerous very small, often coalescing yellow spots, most common and numerous at the tips of the uppermost leaflets of the lower leaves. Gradually the tips of these leaves became rusty. The necrotic spots or rustiness gradually spread over the whole leaf, extending after a time to the leaves higher up on the stem. The check plants remained free from Aucuba mosaic.

The Success plants came up above the ground normal and vigorous and remained for some time absolutely free from any disease symptoms. Gradually during the growing period, there appeared on some of the lower leaves more or less numerous, small, rounded, yellow spots, followed by necrotic

spots. The tops of all ten plants remained normal and free from any disease symptoms until the end of the growing period.

The relation of Aucuba mosaic to net necrosis was apparent at a very early date. At first, however, it was thought that the appearance of Aucuba mosaic on plants from tubers affected with net necrosis might be incidental, as appeared to be the case with leafroll in Schultz and Folsom's work. The constant appearance of Aucuba symptoms on plants from many hundreds of net necrosis tubers from five different sources, and of four different varieties, made it quite improbable that the occurrence of the two diseases on the same plants is incidental.

In order to check the above results, ten healthy Midlothian Early tubers were grafted with ten tubers of the variety May Queen (Koksiaan), originating from plants showing pure Aucuba mosaic. The latter were taken from Prof. Quanjer's collection of virus disease in pure form, from the material which had served for the description of Aucuba mosaic.

The grafted tubers were kept for 50 days at room temperature and were planted on March 23 in well fertilized sandy soil. All plants developed pronounced Aucuba mosaic symptoms. During the second half of June the tubers of all the ten plants showed more or less severe, but in every case distinct, net necrosis when lifted. The neighboring row of check plants showed no Aucuba mosaic and no net necrosis in the tubers.

This experiment shows that even the supposedly pure Aucuba mosaic material used by Quanjer for the description of, and infection work with, this disease transmitted net necrosis to the healthy plants.

SUMMARY

Net necrosis is a tuber symptom, not of leafroll, but of Aucuba mosaic. Spindling sprout, supposed by some to develop on those potato tubers affected with leafroll and with net necrosis, has no relation to leafroll.

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LITERATURE CITED

1. ANONYMOUS. Leaf roll, mosaic, and related diseases of the potato. Scottish Jour. Agr. **8**: 44–55 and 176–189. 1925.
2. COOK, M. T. Potato diseases in New Jersey. N. J. Agr. Exp. Sta. Circ. 105. 1919.
3. COONS, G. H. Michigan potato diseases. Mich. Exp. Sta. Spec. Bul. 85. 1919.
4. DORST, J. C. Knopmutatie bij den aardappel en hare beteekenis voor den landbouw. s'Gravenhage, Martinus Nijhoff 1924. 118 pp. 1924. Dissertation.
5. GILBERT, A. H. The correlation of foliage-degeneration diseases of the Irish potato with various variations of the tuber and sprout. Jour. Agr. Res. **25**: 255–266. 1923. Abstract in Phytopath. **12**: 40. 1922.

6. GÜSSOW, H. T. Report from the division of botany 1914–15 Canada Exp. Farms. Reports 1914–15. 2: 949–990. 1916.
7. JONES, L. R., M. MILLER, and E. BAILEY. Frost necrosis of potato tubers. Wis. Exp. Sta. Res. Bul. 46. 1919.
8. MURPHY, P. A., and R. MCKAY. Investigations on the leafroll and mosaic diseases of the potato. Jour. Dept. Agr. and Tech. Inst. Ireland 23: 344–364. 1924.
9. ORTON, C. R. Potato diseases. Pa. Exp. Sta. Bul. 140: 30–31. 1916.
10. ORTON, W. A. Potato wilt, leafroll, and related diseases. U. S. Dept. Agr. Bul. 64. 1914.
11. QUANJER, H. M. New work on leaf-curl and related diseases in Holland. Rept. Internatl. Potato Conference London 1921: 127–145. Roy. Hort. Soc. 1922.
12. ————————. General remarks on potato diseases of the curl type. Rept. Internatl. Conf. Phytopath. and Econ. Entom. Holland 1923: 23–28. 1923.
13. SCHULTZ, E. S., and D. FOLSOM. Leafroll, net necrosis, and spindling sprout of the Irish potato. Jour. Agr. Res. 21: 47–80. 1921.
14. ————————, ————————. Transmission, variation, and control of certain degeneration diseases of Irish potato. Jour. Agr. Res. 25: 43–117. 1923.
15. ————————, ————————. Infection and dissemination experiments with degeneration diseases of potato. Observations in 1923. Jour. Agr. Res. 30: 493–528. 1925.

BIOCHEMISTRY OF PLANT DISEASES VIII
ALCOHOLIC FERMENTATION OF FUSARIUM LINI[1]

HOUSTON LETCHER and J. J. WILLAMAN

INTRODUCTION

As a continuation of earlier work by Anderson (2) from this laboratory on the biochemistry of *Fusarium lini,* a brief study has been made with regard to alcohol production by the organism. The four principal objects of the present work were the following: (a) to determine the time required for maximum production of alcohol by the organism; (b) to ascertain the alcohol production on different types of saccharine media; (c) to measure the alcohol production of several cultures of the organism, and to correlate, if possible, alcohol production with pathogenicity; and (d) to identify any reaction products of the metabolism of the organism, other than alcohol and carbon dioxide.

With regard to alcohol production by *Fusarium lini,* Anderson (2) reported a value of 3.44 per cent by weight, and that approximately the same percentage of alcohol was toxic to the fungus when cultured on it. This led to the conclusion that the fungus produced alcohol to the point where the concentration became toxic and then used the alcohol so formed as a source of food. Various workers have studied the alcohol production of numerous organisms, but *Fusarium lini* is unique in producing alcohol not only from hexoses but from pentoses as well.

In the course of the present work it was found that *Fusarium lini* gave qualitative tests for acetaldehyde as one of its metabolism products. Neuberg and his associates (6, 7, 8, 9, 10), using $CaSO_3$ and Na_2SO_3 as fixatives [*cf.* Connstein and Luedecke, Ger. Pat. No. 298593/12o. 1915], showed that acetaldehyde was an intermediate compound in fermentations by bacteria and by yeasts.

EXPERIMENTAL METHODS AND MATERIALS

Source of Fusarium lini used. The cultures of *F. lini* used in the present investigation were obtained from the Division of Plant Pathology, Uni-

[1] Published with the approval of the Director as Paper No. 626, Journal Series, Minnesota Agricultural Experiment Station. The paper is abridged from a Master's thesis by the senior author in the University of Minnesota, 1925.

versity of Minnesota, through the courtesy of Dr. Louise Dosdall and Mr. W. C. Broadfoot.[2]

Preparation of media. The composition of the different media are specified in subsequent paragraphs. In general, the media were autoclaved at 10 pounds pressure for 20 minutes. One hundred cc. of liquid were used in a 300-cc. Erlenmeyer flask.

Inoculation. The experimental cultures were inoculated by using small bits of mycelium from well developed stock cultures on liquid media.

Incubation. Culture flasks were kept in incubators at a temperature of 26° to 28° C.

Culture media. Sucrose, 8 and 12 per cent, was used in the following salt-peptone solution:

Monopotassium phosphate	1.00 gm.
Magnesium sulfate	0.25
Ferrous sulfate	0.10
Peptone	7.50

Water to make one liter.

Maltose syrup.—The original syrup of Brix reading 60 was diluted until a reading of 7.6 was obtained.

Corn stalk syrup.—This material was prepared at the Minnesota Experiment Station in 1921. The syrup was diluted to a Brix reading of 11.2.

Beerwort.—This material was furnished through the courtesy of the Minneapolis Brewing Company. Both the original wort of Brix reading 18.45 and diluted portions of Brix 8.0 were used.

Glucose, 8 and 12 per cent, was used in the following salt medium:

KH_2PO_4	0.50 gm.
$MgSO_4$	0.25
NH_4NO_3	1.00

Water to make one liter.

The glucose used was the commercial "Cerelose."

Molasses.—A commercial brand of light New Orleans molasses was diluted to the desired densities.

"Black liquor" from paper mill.—This material was from the Northwest Paper Company, Cloquet, Minnesota. It was classified as "Keebra black liquor from tamarack cook" and had a Brix reading of 34.2. A portion of the liquor dialyzed in a hardened collodion bag for a week was also used.

[2] Broadfoot's results on the cultural characters and pathogenicity of these cultures are given in a separate article in this number of PHYTOPATHOLOGY (Phytopath. 16: 951–978. 1926).

Alcohol determination. One hundred cc. of culture medium was filtered through cheesecloth to remove the mycelium, and the felt was then pressed as dry as possible to remove adhering liquid. Fifty cc. of distilled water was added to the filtrate and the mixture placed in a 1,000-cc. flask connected to a straight tube condenser. The first 100 cc. of distillate were collected and the alcohol in it determined by the specific gravity method, using a chainomatic specific gravity balance.

Qualitative and quantitative tests. The following tests were used on the distillates to determine acetaldehyde, acetone, and alcohol:

Acetaldehyde—Tollen's reaction, according to Mulliken (5).
Schiff's test, according to Weston (12).
The semi-carbazone reaction, as given by Weston (12).
Beta-naphthol test, according to Mulliken (5).
NaOH test, according to Mulliken (5).
Fehling's reaction, according to Allen (1).
Bisulfite precipitation method, originally devised by Ripper and listed by Kingscott and Knight (4).
Piperidine test, according to Neuberg (6).
Acetone—According to method of Bülow (3).
Alcohol—Iodoform, according to the method of Allen (1).

EXPERIMENTAL DATA

Alcohol Production by Two Cultures of Fusarium lini

Forms 7 and 2 were grown on the media shown in tables 1 and 2. The flasks of this series were removed from the incubators at the end of 5, 10, 15, 20, 30, and 40 days and the alcohol determinations made in duplicate. The data are presented in tables 1 and 2 and in figures 1 and 2. It is

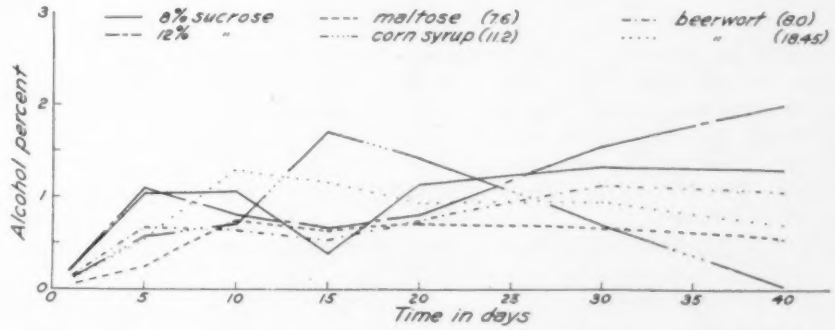

FIG. 1. Production of ethyl alcohol by form 7 of *Fusarium lini* on various media.

TABLE 1.—*Alcohol production by Fusarium lini, form 7*

Media	Age of cultures in days					
	5	10	15	20	30	40
	Alcohol production in per cent					
Sucrose (8 per cent)	1.03	1.07	0.40	1.13	1.33	1.30[a]
Sucrose (12 per cent)	1.09	1.31	0.67	0.80	1.58	2.02
Maltose (Brix 7.6)	0.23	0.73	0.43	0.70	0.67	0.53
Corn syrup (Brix 11.2)	0.57	0.70	1.71	1.44	0.70	0.04
Beerwort (Brix 8.0)	0.67	0.63	0.53	0.73	1.13	1.06
Beerwort (Brix 18.45)	0.53	1.30	1.18	0.93	0.97	0.77

[a] Alcohol determination after 45 days.

apparent that both cultures produce appreciable amounts of ethyl alcohol on all the media; that a maximum is not attained before 20 days or more; that this maximum is usually followed by a decrease; and that form 2 produces more alcohol than form 7. After 10 to 20 days practically all of the cultures had an alcoholic odor, and in the case of the corn stalk syrup cultures the odor was very similar to that of a cider fermentation. The growth of the mycelium was much the same for all the cultures after 20 days, since by that time the mycelium had covered practically all of the available liquid surface and from then on there was an increase in depth of the felts. The good yield of alcohol by form 2 on corn stalk syrup suggested the use of this culture on the syrup in an effort to increase the alcohol and to furnish material for identification trials.

TABLE 2.—*Alcohol production by Fusarium lini, form 2*

Media	Age of cultures in days					
	5	10	15	20	30	40
	Alcohol production in per cent					
Sucrose (8 per cent)	0.57	0.90	0.00[a]	1.72	1.13	1.00
Sucrose (12 per cent)	0.53	1.51	2.50	3.27	3.24[b]	2.05
Maltose (Brix 7.6)	0.50	1.68	0.97	1.44	0.66	1.19
Corn syrup (Brix 11.2)	0.77	3.17	3.52	3.34	2.22	2.19
Beerwort (Brix 8.0)	0.46	1.10	1.33	0.67	1.40	1.03
Beerwort (Brix 18.45)	0.63	1.23	1.95	1.47	2.68	2.61
Glucose (8 per cent)	0.46	1.23	2.40	2.47	1.07	0.80
Glucose (12 per cent)	0.50	0.93	1.95	2.64	3.31	2.99

[a] No growth of mycelium.

[b] Alcohol determination after 25 days.

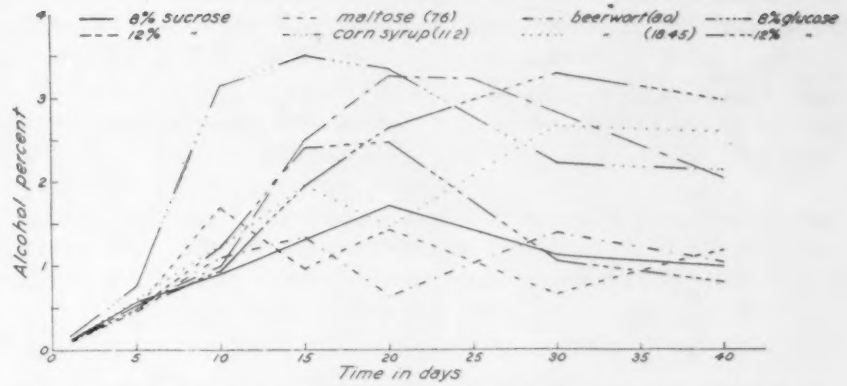

FIG. 2. Production of ethyl alcohol by form 2 of *Fusarium lini* on various media.

Alcohol Production by Nine Cultures of Fusarium lini on Molasses

To determine the differences in alcohol production among several cultures of *Fusarium lini* on a single medium, 108 flasks were prepared, using molasses of Brix reading 11.8 to 12.1. The flasks were incubated from 5 to 30 days, and every fifth day two samples in each series were removed from the incubators for alcohol determinations. The results are given in table 3 and in figure 3.

Forms 6 and 9 form a group with a relatively low alcohol production, while the others yielded high percentages of alcohol. Table 3 gives data

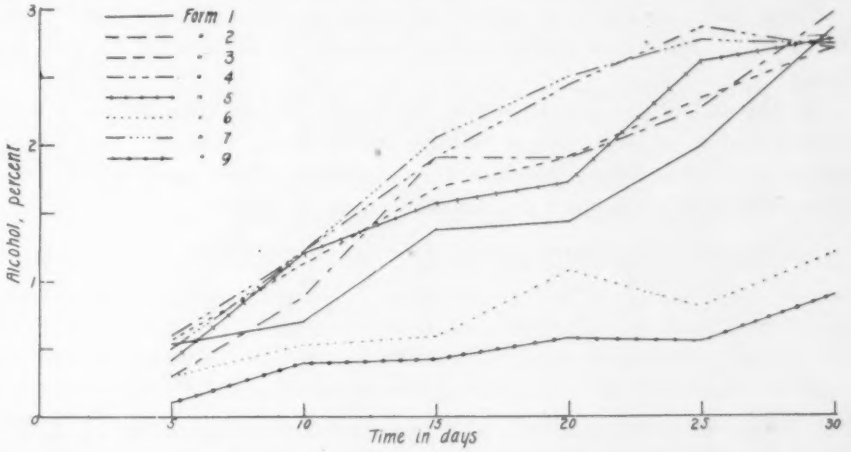

FIG. 3. Production of ethyl alcohol by various forms of *Fusarium lini* on a molasses medium.

TABLE 3.—*Alcohol production by forms of Fusarium lini on molasses*

Form used	Rank in virulence on host	Rank in alcohol production at 20 days	Age of cultures in days					
			5	10	15	20	25	30
			Alcohol production in per cent					
1	1	6	0.53	0.70	1.37	1.44	1.98	2.85
2	2	3	0.57	1.13	1.68	1.92	2.33	2.71
3	3	4	0.30	0.90	1.92	1.92	2.29	2.99
4	4	2	0.70	1.23	1.92	2.43	2.85	2.71
5	5	5	0.43	1.20	1.57	1.72	2.61	2.78
6	6	7	0.30	0.53	0.60	1.17	0.83	1.24
7	7	1	0.50	1.23	2.06	2.50	2.77	2.75
9	8	8	0.23	0.40	0.43	0.59	0.57	0.90

relative to the virulence of cultures of the fungus on flax. From these data it is evident that forms 6 and 9, with the lowest alcohol production, are also the least virulent.

Identification of End Products

To obtain larger quantities of fermentation products and to use this material for identification work, the following series of flasks were prepared:

Series A	15	flasks	10	days incubation
B	18	do	20	do
C	17	do	30	do

These flasks were kept in a room the temperature of which ranged from 20° to 31° C., but which approximated 26° to 28° C. the greater part of the time.

At the end of the growth period 500 cc. of filtrate from Series A was fractionated in a flask with a 14-inch fractionating column. One 50-cc. portion and three 100-cc. portions were obtained. Three such 500-cc. portions were fractionated and the following data obtained:

Fraction of distillate	Alcohol percentages		
	I	II	III
50 cc.	25.03	25.31	13.81
100 cc.	0.00	0.00	0.80
100 cc.	0.00	0.00	0.00
100 cc.	0.00	0.00	0.00

The 100-cc. portions were run to determine if it were possible to obtain fractions of specific gravity greater than 1.0000. A blank determination on the corn stalk syrup which had been autoclaved but not inoculated gave three 100-cc. portions of specific gravity 1.0000. The three 50-cc. portions

were combined and fractionated. The first 45 cc. of distillate had an alcohol content of 61.6 per cent, coming off largely at 78°–97° C. The second 45-cc. portion distilled at 97°–98.2° C. but principally at 98° C., and had an alcohol percentage of 1.13. The first fraction had the odor of a yeast fermentation, and was acid to litmus. It gave the iodoform test on heating, as well as in the cold, indicating the possibility of aldehyde. The latter was further shown by a positive fuchsin test.

The filtrate from the 18 flasks in Series B, 20 days old, was fractionated as in Series A. The first 50-cc. fractions (from 500 cc. of filtrate) were combined as above and distilled. The first 50 cc. boiled at 78°–79.2° C. and had an alcohol percentage of 89.0; the second 50 cc. contained 25.70 per cent alcohol; and the third, 0.00. On applying analytical tests for aldehyde to the first of these the following results were obtained:

Fuchsin aldehyde reagent	—	positive for aldehyde
Tollen's reaction	—	do
Fehling's reaction	—	doubtful for acetaldehyde
Beta naphthol test	—	negative do
NaOH test	—	positive do
Bisulphite titration	—	do do

As to the Fehling's reaction, a precipitate of copper was obtained both in the blank and in the test solutions, and the difference in quantity of the precipitates was not great enough to justify the test as positive for acetaldehyde. Mulliken (5) states that the beta naphthol test is not applicable to small amounts of the aldehyde, and the evidence obtained here was that there was but a very small amount of the acetaldehyde present in the distillates. The bisulfite titration showed 0.0064 gm. of acetaldehyde per 100 cc. of distillate.

In Series C the first fraction had an alcohol percentage of 77.75 and the bisulfite titration showed 0.0457 grams of acetaldehyde per 100 cc. of distillate.

Further Studies on End Products of Fermentation

Because of the volatility of acetaldehyde, the following distillations were carried out with the precautions suggested by Neuberg, Hirsch and Reinfurth (10). The fractionating flask was connected to a straight tube Liebig condenser and this joined by an adapter to a vertically placed coil condenser; the latter was connected by an adapter to two suction flasks connected in series, with the second flask as a safety. The flasks were immersed in an ice-bath. Thus every effort was made to obtain complete condensation of the distillates. Fifty cc. of distillate, fractionated from the filtrate from 30 culture flasks, gave an alcohol percentage of 89.05. Since

earlier tests had shown the possibility of acetone or acetaldehyde as a fermentation product, these substances were tested for in this 50-cc. fraction, as follows:

Acetone test—negative
Piperidine test—positive for acetaldehyde
Semi-carbazone reaction—positive for acetaldehyde

Tollen's reaction, NaOH test, and the fuchsin aldehyde reagent were all positive for acetaldehyde, as in the earlier tests. The bisulfite titration showed a value of 0.0512 gm. of acetaldehyde per 100 cc. of distillate.

The foregoing qualitative tests are of interest in view of the statement of Stepp (11) that the Tollen's reaction is positive for 1 part of acetaldehyde in 100,000. Furthermore, Stepp mentioned that the fuchsin aldehyde reagent is not very sensitive and that acetone did not give a color with it. Hence the facts that a good color was obtained with the fuchsin aldehyde reagent, that acetone does not give any reaction with the piperidine reagent and that the acetone test of Bülow was negative indicated the probable absence of acetone as a fermentation product. Since Stepp mentioned that the piperdine reaction was not given by formaldehyde or benzaldehyde, the latter were probably not present to interfere with the bisulfite titration. From the foregoing tests it was concluded that acetaldehyde is probably one of the fermentation products of *Fusarium lini*. The use of $CaSO_3$ as a fixative agent, as devised by Neuberg, should establish definitely whether or not acetaldehyde is an intermediate fermentation product.

Growth of Cultures of Fusarium lini on Paper Mill Waste Liquor

Three 100-cc. portions of the waste liquor from a paper mill were inoculated with forms 1 and 2, and incubated for 35 days at 26°–28° C. The liquor had a Brix reading of 34.2, but there was no growth of the fungus after 35 days. This trial was repeated with forms 2, 3, 6 and 7, but no growth resulted after 15 days incubation. Twenty cc. portions of the liquor which had been dialyzed were inoculated with forms 2, 3 and 7. Some mycelium developed after a 12-day incubation period. This result shows further the omnivorous nature of this fungus.

SUMMARY

1. *Fusarium lini*, the fungus causing flax wilt, has been cultured on six different saccharine media, and produced ethyl alcohol on all of them. This confirms the earlier observations of Anderson (2).

2. In general the maximum alcohol content of the liquid cultures was attained in approximately 30 days.

3. The alcohol production by eight forms of the fungus ranged from 0.80 to 2.99 per cent by volume 30 days after inoculation. The highest percentage for any single culture was 3.7.

4. The two forms of the organism which were the least virulent in their action on the flax plant had the lowest alcohol production.

5. Acetaldehyde apparently is one of the intermediate products formed by the fungus in fermenting sugars.

6. The acetaldehyde so derived was present at any given time only in traces, as shown by color and titrimetric determinations.

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LITERATURE CITED

1. ALLEN. Commercial organic analysis. Vol. I. Blakiston. 1909.
2. ANDERSON, A. K. Biochemistry of plant diseases, VI. Biochemistry of *Fusarium lini* Bolley. Minn. Studies in Plant Science. No. 5. 237–280. 1924.
3. BÜLOW, C. A new method of detecting and estimating small amounts of acetone. Science **61**: 344. 1925.
4. KINGSCOTT, P. R. C. and R. S. G. KNIGHT. Methods of quantitative analysis. London. 1914.
5. MULLIKEN, S. P. A method for the identification of pure organic compounds. Vol. I. Edition I. Wiley. 1905.
6. NEUBERG, C. Detection of acetaldehyde in alcoholic fermentation. Woch. Brau. **36**: 292. 1919. Abst. in Chem. Abst. **14**: 2235. 1919.
7. NEUBERG, C. and F. F. NORD. Anwendung der Abfangmethode auf die Bakteriengärungen, I. Acetaldehyde als Zwischenstufe bei der Vergärung von Zucker, Mannit und Glycerin durch *Bacterium coli*. Biochem. Ztschr. **96**: 133–157. 1919.
8. NEUBERG, C. and F. F. NORD. Anwendung der Abfangmethode auf die Bakteriengärungen, II. Festlegung der Aldehydstufe bei der Essiggärung. Biochem. Ztschr. **96**: 158–174. 1919.
9. NEUBERG, C., F. F. NORD, and E. WOLF. Acetaldehyde als Zwischenstufe bei der Vergärung von Zucher durch *B. lactis aerogenes*. Biochem. Ztschr. **112**: 144–150. 1920.
10. NEUBERG, C., J. HIRSCH, and E. REINFURTH. Weitere Mitteilungen uber die äquivalente Bildung von Acetaldehyde und Glycerin bei der zweiten Vergärungsform. Biochem. Ztschr. **132**: 589–596. 1922.
11. STEPP, W. Zur Frage des Acetaldehydnachweises im Harn. Biochem. Ztschr. **127**: 13–17. 1922.
12. WESTON, F. E. A scheme for the detection of the more common classes of carbon compounds. Edition III. New York. 1912.

STUDIES ON THE PARASITISM OF FUSARIUM LINI BOLLEY[1]

W. C. Broadfoot

The development and distribution of resistant varieties of flax has aided materially in the solution of the flax wilt problem. However, resistant varieties are not always resistant. They may be resistant in one locality and not in another. This difference in resistance may be due partly to environmental conditions, as Tisdale (10) and Barker (4) have shown that wilt resistance is only relative and may be modified by environmental conditions. It has been demonstrated that the effect of environment may be overcome to some extent by early planting (4). However, the results given in a former abstract (6) and in this paper indicate that the differences in reaction of resistant varieties in different localities may be due also to physiologic specialization of the pathogene. If this is true, it will be necessary either to select resistant varieties for certain localities, or varieties resistant to all physiologic forms.

Since Tisdale (11), Barker (4), and Anderson (1) have reviewed the literature on flax wilt, it will be unnecessary to review it again in this paper.

Five varieties of flax, namely, N. D.[2] 40013 (C. I. 241); N. D. 3080 (C. I.

Fig. 1. Reaction to *Fusarium lini*, form 1, on Primost, Minn. 25 (C. I. 177), in pots. Check pot on the right.

[1] Published with the approval of the Director as paper No. 657 of the Journal Series of the Minnesota Agricultural Experiment Station.

The writer desires to acknowledge his indebtedness to Dr. E. C. Stakman for his helpful advice and criticism.

[2] N. D. = North Dakota Agricultural Experiment Station accession number.

C. I. = Office of Cereal Investigations accession number.

Minn. = Minnesota Agricultural Experiment Station accession number.

275); Slope (C. I. 274); Winona, Minn. 182 (C. I. 179); and Chippewa, Minn. 181 (C. I. 178), when grown at Mandan, N. D., were highly resistant to flax wilt and yielded well, according to data of Stoa and Dillman (9). When grown at University Farm, St. Paul, Minn.,[3] on the other hand, the first three varieties were more or less susceptible to wilt and yielded less than the other two, which were highly resistant also at University Farm. Table 1 shows the striking differences in yields when the varieties were

TABLE 1.—*Yields in bushels per acre of flax varieties grown at Mandan, North Dakota, and University Farm, St. Paul, Minnesota*

Flax varieties	C.I.[a]	Yields in bushels per acre	
		Mandan, N. Dak.	Univ. Farm, St. Paul, Minn.
N. D. 40013	241	11.5	1.89
N. D. 3080	275	11.3	1.31
Slope	274	10.1	3.19
Winona, Minn. 182	179	10.4	9.01
Chippewa, Minn. 181	178	10.2	10.20

[a] C. I. = Office of Cereal Investigations accession number.

grown at University Farm, St. Paul, Minn., and the lack of significant differences at Mandan, N. D.

Inasmuch as there seemed to be strong circumstantial evidence of physiologic specialization in *Fusarium lini*, the writers attempted to ascertain whether the fungus actually was specialized, and to determine the number of forms, their morphologic characteristics, parasitic capabilities, degree of stability, and mode of action.

MATERIALS AND METHODS

Table 2 lists the sources of material for cultures of *F. lini*. Four varieties of flax (*Linum usitatissimum* L.), namely, Primost, Minn. 25 (C. I. 177); Winona, Minn. 182 (C. I. 179); N. D. 3080 (C. I. 275); and N. D. 40013 (C. I. 241) were used as differential hosts for inoculation experiments. The varieties were supplied by Dr. A. W. Henry, of the Division of Plant Pathology, University of Minnesota. Cultures, accession Nos. 1, 2, 4, to 17, inclusive, are of monosporous origin. These were obtained by picking up single germinating spores with a sterile platinum needle from poured agar plates. In the case of culture No. 3, a single chlamydospore-like body in a hyphal thread was picked up. Stock cultures were grown on potato dextrose agar and were kept at room temperature.

[3] Unpublished results furnished by Doctor A. W. Henry, University Farm, St. Paul, Minnesota.

TABLE 2.—*Source of materials for cultures of Fusarium lini isolated from flax*

Accession no.	Locality or origin	Transferred from	Collector or sender	Isolated by	Date isolated or transferred
1	Agronomy plot, St. Paul, Minn.		W. C. Broadfoot	W. C. Broadfoot	Aug. 2, 1924
2	Austin, Minn.		J. J. Christensen	W. C. Broadfoot	July 24, 1924
3	Cadillac, Sask.		R. G. Olgivie	W. C. Broadfoot	Nov. 6, 1924
4	Cadillac, Sask.		R. G. Olgivie	W. C. Broadfoot	Nov. 6, 1924
5	Crookston, Minn.		A. W. Henry	W. C. Broadfoot	Sept. 2, 1924
6	Crookston, Minn.		A. W. Henry	W. C. Broadfoot	Sept. 2, 1924
7	Fargo, N. D.	Minn. Herb. culture no. 53	H. D. Barker	H. D. Barker July 5, 1921	Aug. 30, 1924
8	Kenyon, Minn.	Minn. Herb. culture no. 52	H. D. Barker	H. D. Barker March 11, 1921	Aug. 30, 1924
9	Morris, Minn.		J. J. Christensen	W. C. Broadfoot	July 24, 1924
10	Plant Pathology plot, St. Paul, Minn.		W. C. Broadfoot	W. C. Broadfoot	Aug. 2, 1924
11	Red Lake Falls, Minn.		A. W. Henry	W. C. Broadfoot	Aug. 20, 1924
12	Saskatoon, Sask.		G. Boyd	W. C. Broadfoot	Nov. 6, 1924
13	St. Lawrence, Minn.		A. W. Henry	W. C. Broadfoot	Aug. 2, 1924
14	Winnipeg, Man.		G. R. Bisby	W. C. Broadfoot	Sept. 30, 1924
15	Winnipeg, Man.		G. R. Bisby	W. C. Broadfoot	Sept. 30, 1924
16	St. Paul, Minn.	Minn. Herb. culture no. 58	H. D. Barker	H. D. Barker May 20, 1921	Sept. 30, 1924
17	do	Minn. Herb. culture no. 60	H. D. Barker	H. D. Barker Nov. 25, 1921	Sept. 30, 1924
19	do	Minn. Herb. culture no. 95	O. S. Aamodt	O. S. Aamodt July, 1915	Mar. 6, 1925
20	do	Minn. Herb. culture no. 16	H. D. Barker	H. D. Barker 1919	Mar. 6, 1925

TABLE 3.—*Results of inoculating four varieties of flax with nine physiologic forms of Fusarium lini*

Physiologic form	Accession no.	Trial	Varieties and Reactions[a]											
			Primost, Minn. 25				Winona, Minn. 182				N. D. 3080			
			Surviving plants			Reaction	Surviving plants			Reaction	Surviving plants			Reaction
			Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials		Total no.	Percent	Pct. of two trials	
1	3	1	0	0.0		C	0	0.0		C	3	3.7		C
		2	0	0.0	0.0	C	0	0.0	0.0	C	0	0.0	1.9	C
2	7	1	2	2.0		S	7	10.9		S	8	10.0		S
		2	0	0.0	1.0	S	1	1.8	6.4	S	0	0.0	5.0	S
3	9	1	0	0.0		S	4	6.2		S	16	20.0		R
		2	2	3.8	1.9	S	9	17.9	11.5	S	16	27.5	23.5	R
4	8	1	6	5.7		S	23	36.6		R	5	6.3		S
		2	12	16.3	11.0	S	37	73.6	55.1	R	6	10.3	8.2	S
5	2	1	7	6.6		S	1	1.4		S	10	12.2		S
		2	4	7.1	6.8	S	2	4.2	2.8	S	2	3.9	7.9	S
6	6	1	11	10.5		S	15	23.8		R	3	3.7		S
		2	5	9.3	9.9	S	23	46.0	34.9	R	8	13.5	8.6	S
7	4	1	6	5.7		S	18	28.6		R	26	32.2		R
		2	9	16.4	11.0	S	26	52.1	40.4	R	22	37.8	35.0	R
8	5	1	25	23.7		R	39	61.9		R	11	13.5		S
		2	25	45.3	34.5	R	24	47.9	59.9	R	0	0.0	6.8	S
9	1	1	54	56.2		R	33	52.4		R	67	76.6		R
		2	19	34.4	45.3	R	12	23.9	38.1	R	19	32.6	54.6	R
Uninoculated check		1	106	100.0			61	100.0			81	100.0		
		2	55	100.0	100.0		50	100.0	100.0		58	100.0	100.0	

Physiologic form	Accession no.	Trial	N. D. 40013			
			Surviving plants			Reaction
			Total no.	Percent	Pct. of two trials	
1	3	1	1	1.0		C
		2	0	0.0	0.5	C
2	7	1	9	9.8		S
		2	4	7.2	8.5	S
3	9	1	0	0.0		S
		2	10	12.4	6.2	S
4	8	1	17	18.8		S
		2	10	18.3	18.5	S
5	2	1	28	30.3		R
		2	19	35.0	32.6	R
6	6	1	38	41.8		R
		2	18	33.6	37.7	R
7	4	1	11	12.2		S
		2	5	9.4	10.8	S
8	5	1	11	13.5		S
		2	0	0.0	6.8	S
9	1	1	21	23.1		R
		2	30	55.5	39.3	R
Uninoculated check		1	91	100.0		
		2	54	100.0	100.0	

[a] R = Resistant; S = Susceptible; C = Susceptible and chlorotic above cotyledons.

The inoculum for soil inoculations was produced on a sterilized medium consisting of a mixture of sterilized wheat, oats, and barley seeds combined with an equal volume of water. An equal amount of inoculum of each culture was mixed with sterilized soil in twelve 4-inch pots. Seeds of differential hosts were surface disinfected by immersion for 10 minutes in a 1-1000 $HgCl_2$ solution, then washed in sterile distilled water and allowed to dry. In each pot in experiment 1, 50 seeds of one variety were sown, and 25 seeds per pot in experiment 2. The control pots were treated in the same way, except that sterilized uninoculated medium was mixed with the soil. Two inoculation experiments, each in triplicate, were made in the greenhouse, one in March and the other in June. The number of plants surviving in the control pots was considered as 100 per cent. Varieties were arbitrarily designated resistant if more than 20 per cent of the plants survived.

RESULTS

Various degrees of resistance and susceptibility appeared. On this basis nine physiologic forms of *F. lini* were recognized. The results of the inoculations with nine forms are summarized in table 3.

An examination of table 3 shows that forms 1 and 2 are somewhat similar in pathogenicity. However, form 1 causes the plants to lose their chlorophyll, a condition which is brought about by no other form (Fig. 1). When the plants are from 4 to 6 inches high the green color gradually disappears from all the tissues above the cotyledons until this portion of the plant eventually becomes entirely colorless. The line of demarcation is very sharp between the normal and "chlorophyll deficient" tissues (Fig. 2). Sections of these tissues show that the chloroplasts are decidedly deformed as well as colorless. The affected plant eventually dies. This condition was produced by artificial inoculation in the field as well as in the greenhouse. Form 1 also caused a similar condition on Manchuria barley, Victory oats, and Rosen rye. The green color gradually disappeared from all the tissues above the first leaf.

The forms are numbered in order of their virulence. All four varieties are susceptible to forms 1 and 2, which are the most virulent. Only one variety, a different one for each of the forms, is resistant to forms 3, 4, and 5. Similarly, two varieties are resistant to forms 6, 7, and 8. Form 9 is the least virulent. All four varieties are resistant to this form, although it kills some of the plants of all varieties. For convenience in distinguishing physiologic forms of *F. lini*, an analytical key of the simple dichotomous type is presented in table 4.

Table 5 gives the place of collection of the nine forms of *F. lini* used in the experiments. No attempt was made to determine the prevalence and

TABLE 4.—*Analytical key to physiologic forms of Fusarium lini*

Chlorosis absent		
Primost, Minn. 25 (C. I. 177)	Resistant	
N. D. 3080 (C. I. 275)	Resistant	Form 9
N. D. 3080	Susceptible	Form 8
Primost	Susceptible	
N. D. 3080	Resistant	
Winona, Minn. 182 (C. I. 179)	Resistant	Form 7
Winona	Susceptible	Form 3
N. D. 3080	Susceptible	
N. D. 40013 (C. I. 241)	Resistant	
Winona	Resistant	Form 6
Winona	Susceptible	Form 5
N. D. 40013	Susceptible	
Winona	Resistant	Form 4
Winona	Susceptible	Form 2
Chlorosis present		Form 1

distribution of the different forms. However, certain of the forms studied appeared to be more widely distributed than others. Thus forms 2, 3, and 7 were found both in the United States and Canada, while form 1 was found only in Saskatchewan and the other forms in Minnesota alone. But it must be borne in mind that only very few collections were made, and further studies might show the situation to be entirely different.

TABLE 5.—*Place of collection of nine physiologic forms of Fusarium lini*

Accession no.	Physiologic form	Place collected
3	1	Cadillac, Saskatchewan
7	2	Fargo, North Dakota
12		Saskatoon, Saskatchewan
13		St. Lawrence, Minnesota
20		University Farm, St. Paul, Minnesota
9	3	Morris, Minnesota
14		Winnipeg, Manitoba
15		Winnipeg, Manitoba
8	4	Kenyon, Minnesota
2	5	Austin, Minnesota
19		University Farm, St Paul, Minnesota
6	6	Crookston, Minnesota
11		Red Lake Falls, Minnesota
4	7	Cadillac, Saskatchewan
17		University Farm, St. Paul, Minnesota
5	8	Crookston, Minnesota
1	9	Agronomy plot, University Farm, St. Paul, Minnesota
10		Plant Pathology plot, University Farm, St. Paul, Minnesota

MORPHOLOGY OF THE PHYSIOLOGIC FORMS OF FUSARIUM LINI

The results of these experiments indicate clearly that there are physiologic forms of *F. lini*. Experiments were then made to determine whether there are differences in morphology as well as in pathogenicity. The nine forms of *F. lini* were grown on potato plugs, steamed rice, hard oat agar, sweet clover stems, alfalfa stems, 2 per cent potato dextrose agar, and 5 per cent potato dextrose agar, all in test tubes, according to the formulae recommended by Wollenweber, Sherbakoff, Reinking, Johann, and Bailey (12) for the identification of species of *Fusarium*. *F. lini* was first described by Bolley (5) from cultures grown on slightly acid peptone agar. Such an agar was made, consisting of 2 grams of peptone, 2 grams of sugar, 2 grams of agar, a few drops of lactic acid, and 1,000 cc. of water.

Spore measurements were made with an eye-piece micrometer. A 10 per cent aqueous solution of glycerine was used for mounting the spores on the glass slides.

Comparisons of mean length and width of different magnitude of spore populations of Fusarium lini, form 1, grown in the same medium

Separate spore populations of 50, 100, and 200 spores of *F. lini*, form 1, grown on 5 per cent potato dextrose agar 56 days old, were measured for length and width. From the data thus obtained, biometric constants were calculated by the assumed mean method (3). They are given in table 6. The differences in mean length and width of each spore population were then compared in relation to their probable errors (table 7). The differences between lengths of 50 spore and those of 100 and 200 spore populations are more than three times the probable error of the difference. When the mean length of 100 spores is compared with that of 200, the difference in the two means is less than one times the probable error of the difference. It would seem, therefore, that 50 spores may not constitute a representative random sample for drawing conclusions, but that 100 spores will probably be sufficient for a comparison of the spore lengths of *F. lini*. Similarly, it appears that measurements of 50 spores might be sufficient to determine spore width. However, in all cases of further comparative spore measurements, 100 spores were individually measured for length and width.

Comparisons of the length and width of 100 spores of Fusarium lini, form 1, grown on different media

Measurements were made of 100 spores of form 1 grown on 3 different media and are listed in table 8. In one case the spores were taken from a culture of form 1, 14 days old, grown on slightly acid peptone agar. The cultures grown on sweet clover stems and on 5 per cent dextrose agar were 56 days old. The mean length of the spores from the 5 per cent potato dex-

TABLE 6.—*Variations and constants for length and width of spores of Fusarium lini, form 1, based on measuring populations of different magnitudes, under similar conditions*

Medium	Age of culture in days	Magnitude of population samples	Spore classes according to length in microns						Constants			Spore classes according to width in microns						Constants		
			20	25	30	35	40	45	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error	3.0	3.3	3.6	3.9	4.2	4.5	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
5 pct. potato dextrose agar	56	50	8	16	11	10	4	1	28.90 ± 0.61	6.35 ± 0.43	21.97 ± 1.38	4	15	13	10	5	3	3.64 ± 0.04	0.40 ± 0.03	10.98 ± 0.74
do	56	100	33	34	16	10	4	3	26.35 ± 0.44	6.57 ± 0.31	24.93 ± 1.19	4	39	28	21	5	3	3.58 ± 0.02	0.33 ± 0.02	9.22 ± 0.44
do	56	200	53	78	43	16	9	1	26.38 ± 0.27	5.65 ± 0.19	21.40 ± 0.77	9	78	74	27	8	4	3.54 ± 0.01	0.30 ± 0.01	8.47 ± 0.28

TABLE 7.—*Summary of comparisons between means and coefficients of variability for length and width of 100 spores of Fusarium lini, form 1, based on data in table 6*

Conditions compared: magnitude of population sample	Length				Width			
	Means		Coefficient of variability		Means		Coefficient of variability	
	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference
50 and 100	2.55 ± 0.75	3.40	2.96 ± 1.90	1.50	0.06 ± 0.04	0.13	1.76 ± 0.86	2.04
50 and 200	2.52 ± 0.66	3.80	0.56 ± 1.65	0.33	0.10 ± 0.04	2.50	2.51 ± 0.71	3.16
100 and 200	0.03 ± 0.52	0.05	0.47 ± 1.39	0.33	0.04 ± 0.03	1.53	0.75 ± 0.52	1.43

TABLE 8.—*Variations and constants for length and width of 100 spores of Fusarium lini, form 1, on different media*

Medium	Age of culture in days	Spore classes according to length in microns							Constants			Spore classes according to width in microns						Constants		
		15	20	25	30	35	40	45	Mean and probable error	Standard deviation and prob-	Coefficient of variability and probable error	3.0	3.3	3.6	3.9	4.2	4.5	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
5 pct. potato dextrose agar	56		33	34	16	10	4	3	26.35 ± 0.44	6.57 ± 0.31	24.93 ± 1.19	4	39	28	21	5	3	3.58 ± 0.02	0.33 ± 0.02	9.22 ± 0.44
Sweet clover	56	24	44	20	10	1	1		21.15 ± 0.34	5.09 ± 0.24	24.06 ± 1.15	10	51	29	7	3		3.43 ± 0.02	0.26 ± 0.01	7.63 ± 0.36
Peptone agar	14		8	28	33	22	7	2	29.10 ± 0.38	5.66 ± 0.27	19.50 ± 0.93	8	34	29	21	8		3.56 ± 0.02	0.33 ± 0.02	9.30 ± 0.44

TABLE 9.—*Summary of comparisons between means and coefficients of variability for length and width of 100 spores of Fusarium lini, form 1, based on data in table 8*

Conditions compared: media	Length				Width			
	Means		Coefficient of variability		Means		Coefficient of variability	
	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference	Difference and probable error	Difference ÷ the probable error of the difference
5 pet. potato dextrose agar and sweet clover	5.20 ± 0.55	9.29	0.87 ± 1.65	0.52	0.15 ± 0.03	5.35	1.59 ± 0.57	2.77
5 pet. potato dextrose agar and peptone agar	2.75 ± 0.58	4.70	5.43 ± 1.51	3.59	0.02 ± 0.02	0.83	0.08 ± 0.68	0.12
Sweet clover and peptone agar	7.95 ± 0.51	15.49	4.56 ± 1.48	3.09	0.13 ± 0.03	4.64	1.67 ± 0.57	2.91

trose culture was found to be 26.35 ± 0.44 microns; for the spores from sweet clover culture the mean length was 21.15 ± 0.34 microns. The difference between these means is more than nine times the probable error of the difference, which apparently is quite significant. The difference in the mean width of spore cultures grown on 5 per cent potato dextrose agar and on the sweet clover was found to be more than five times the probable error of the difference. This again apparently is significant. The difference in the mean length and width of spores from cultures grown on sweet clover

FIG. 2. Reaction to *Fusarium lini*, form 1, on individual plants of Primost, Minn. 25 (C. I. 177), taken from inoculated soil. Plants on the right are from the check pots.

and from those grown on slightly acid peptone agar were found to be respectively more than fifteen and four times the probable error of the difference. These differences also are apparently very significant, certainly with respect to the mean length. The difference in mean length and width of spores from cultures grown on 5 per cent potato dextrose agar and slightly acid peptone agar are respectively more than four times and less than one times the probable error of the difference. The difference in mean length is apparently significant; whereas the difference in width probably is not. These results are summarized in table 9. Larger or smaller differences might have been obtained if spores had been grown on other media and similarly compared. Such differences as these undoubtedly account for conflicting data on spore size given by different authors for the same organism. It is evident that there may be a marked and significant difference in spore size of a given form of *F. lini* when grown on several different media. Therefore it is important to maintain uniform cultural conditions with respect to media and environment when the comparative morphology of forms of *F. lini* is studied.

Comparisons of the mean length and width of 100 spores of nine forms of Fusarium lini from cultures grown in the same medium

Table 10 gives a summary of the measurements of nine forms from cultures 7 to 14 days old grown on slightly acid peptone agar. Form 5 was measured with a screw micrometer. The mean length varied from 20.10 ± 0.33 microns for form 7, to 35.95 ± 0.42 microns for form 5. The mean width varied from 3.27 ± 0.03 microns for form 2, to 4.08 ± 0.04 microns for form 5. These differences are apparently significant. However, forms 2 and 7 have mean lengths of 20.70 ± 0.27 microns and 20.10 ± 0.33 microns respectively; in this case the differences are smaller. Greater differences might have occurred if thousands of spores had been measured. Therefore spore size alone is not a sufficient basis for a definite separation of forms of *F. lini*.

It has been shown that the same form of *F. lini* differs in spore size when grown on different media. On the same medium, some of the forms differ significantly, while others do not. Therefore spore size of forms of *F. lini* is not an accurate criterion for separating them into distinct morphological categories; but it indicates that there are inherent differences in the spore dimensions of different physiologic forms when cultured on the same medium under identical environmental conditions.

Comparison of cultural and structural characteristics of nine forms of Fusarium lini grown on seven different media

Tables 11 to 17 inclusive summarize the structural and cultural characteristics of nine forms of *F. lini* cultured on seven media in test tubes, as

TABLE 10.—*Variations and constants for length and width of 100 spores of each of the nine physiologic forms of Fusarium lini grown on slightly acid peptone agar*

Accession no.	Spore classes according to length in microns								Constants			Spore classes according to width in microns									Constants		
	15	20	25	30	35	40	45	50	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error	2.7	3.0	3.3	3.6	3.9	4.2	4.5	4.8	5.1	Mean and probable error	Standard deviation and probable error	Coefficient of variability and probable error
3		8	28	33	22	7	2		29.10 ± 0.38	5.66 ± 0.27	19.50 ± 0.93		8	34	29	21	8				3.56 ± 0.02	0.33 ± 0.02	9.30 ± 0.44
7	19	54	22	4	1				20.70 ± 0.27	4.00 ± 0.19	19.30 ± 0.92		6	26	32	28	8				3.62 ± 0.02	0.31 ± 0.02	8.56 ± 0.41
9	2	17	57	22	2				25.25 ± 0.25	3.70 ± 0.18	14.65 ± 0.70		20	16	41	10	6	5	2		3.27 ± 0.03	0.43 ± 0.02	13.14 ± 0.63
8		13	24	29	32	2			29.30 ± 0.40	5.33 ± 0.25	18.19 ± 0.87		2	18	36	27	16	1			3.42 ± 0.02	0.46 ± 0.02	13.45 ± 0.64
2		2	7	22	24	30	14	1	35.95 ± 0.42	6.29 ± 0.30	17.50 ± 0.83	2	4	11	8	27	9	27	8	4	4.08 ± 0.04	0.61 ± 0.03	15.00 ± 0.82
6	4	44	48	2	1	1			23.75 ± 0.25	3.70 ± 0.18	15.60 ± 0.74			16	46	33	4	1			3.68 ± 0.02	0.24 ± 0.01	6.52 ± 0.31
4	37	31	28	2	1	1			20.10 ± 0.34	4.95 ± 0.24	24.60 ± 1.17		4	47	37	11	1				3.47 ± 0.02	0.23 ± 0.01	6.63 ± 0.32
5		1	37	50	11	1			29.70 ± 0.44	3.65 ± 0.31	12.30 ± 0.59			20	44	31	4	1			3.67 ± 0.02	0.25 ± 0.01	6.81 ± 0.33
1		22	39	23	12	2	2		26.95 ± 0.38	5.65 ± 0.27	21.00 ± 1.00		3	28	31	32	4	1	1		3.64 ± 0.01	0.10 ± 0.01	2.75 ± 0.13

recommended by Wollenweber *et al* (12). The percentages of spores falling in the septation classes, as listed, were obtained by examination of 100 spores as practiced by Appel and Wollenweber (2) and Sherbakoff (8). The spore sizes are given on the basis of an average of 10 macroconidia, individually measured for length and width. Spore dimensions were not given for less than 10 spores. The presence or absence of microconidia, macroconidia, terminal and intercalary chlamydospores, the color of mycelium, and substrate was quite variable. Sporulation was not common for all the forms on any one of these media. Forms 1 and 6 sporulated on only two of the media; form 4 on three media; forms 3, 5, 7, and 8 on four media; form 2 on five of the media; and form 9 on seven of the media. There was also great variation in other microscopical characters.

TAXONOMIC POSITION OF FUSARIUM LINI IN FORM GENUS FUSARIUM

Some of the characteristics common to section Elegans (12) of form genus *Fusarium* and *F. lini* are thin-walled microconidia, cylindrical to long ellipsoid, not pyriform, and not catenulate on aerial mycelium. The microconidia are usually present and dominately 0-septate. Macroconidia are usually pedicillate and attenuate at the top end. Terminal and intercalary chlamydospores are usually present. There is no blue or green color in the conidia even as a diffusion from the stroma. The stroma is principally vinaceous to lilac on artificial media.

PHYSIOLOGY OF PHYSIOLOGIC FORMS OF FUSARIUM LINI

Effect of Temperature on Rate of Growth

In order to determine whether the physiologic forms of *F. lini* used in this work could be recognized on the basis of reaction to temperature, a series of tests was made as follows:

Petri dishes of uniform size were poured uniformly with slightly acid peptone agar. At the end of two days, contaminated plates were discarded, and to the others was transferred an approximately uniform amount of mycelium from stock cultures of forms 2, 3, 4, and 5. The plates were incubated at room temperatures for two days before distribution to the various temperature incubators. At the end of 10 days, the diameter of each colony was measured and averaged. The experiment was carried out in duplicate.

The results are illustrated in figure 3. The difference in growth made by the four forms at the various temperatures evidently falls within the limits of experimental error. That is, none of the forms tested could be distinguished from each other on the basis of growth at various temperatures.

TABLE 11.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction[d] of nine physiologic forms of Fusarium lini grown on potato plugs under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Micro-conidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa							
		0	1	2	3		Terminal	Intercalary	
1	3	Macroconidia, if any, rare				–	+	+	30
2	7	5 –	72 20.86 x 3.33	5 –	18 30.64 x 3.33	+	–	–	34
3	9	2 –	7 –	3 –	85 38.2 x 3.52	+	+	+	51
4	8	Macroconidia, if any, rare				–	–	–	51
5	2	9 –	9 –	9 –	73 39.28 x 3.30	+	+	+	30
6	6		Rare			+	+ Rare	+	50
7	4	Macroconidia, if any, rare				–	+	+	43
8	5	80 16.32 x 3.29	19 17.78 x 3.28			+	+	+	48
9	1	93 8.39 x 1.17	7 –						

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the spore size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

TABLE 12.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on hard oat agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa										
		0	1	2	3	4		Terminal	Intercalary		Mycelium	Substrate
1	3	Macroconidia, if any, rare					−	+	+	30	White to light pink	Very light pink
2	7	94 16.32 x 3.26	6 −				+	+	+	43	White	Unchanged
3	9	3 −	7 −	6 −	51 28.86 x 3.39	33 44.66 x 3.36	+	+	+	51	do	do
4	8	19 17.28 x 3.36	35 22.49 x 3.36	18 25.43 x 3.82	28 34.43 x 3.82		+	+	+	51	do	do
5	2	60 28.3 x 3.33	8 −	2 −	30 30.64 x 3.29		+	+	+	30	do	do
6	6		Rare				+	+	+	50	do	do
7	4	23 18.91 x 3.39	35 20.54 x 3.29	6 −	36 30.32 x 3.29		+	+	+	43	do	do
8	5	34 16.63 x 3.29	30 16.63 x 3.52	5 −	31 27.71 x 3.72		+	+	+	48	do	do
9	1	65 7.38 x 1.22	31 6.75 x 1.16	4 −			+	+	+	29	do	do

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the spore size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

TABLE 13.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physio-*

– indicates absence of microconidia or chlamydospores.

TABLE 13.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on 2 per cent potato dextrose agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa						Terminal	Intercalary		Mycelium	Substrate
		0	1	2	3	4						
1	3	Macroconidia, if any, rare					–	+	+	31	White, light pink	Light pink
2	7	25 17.28 x 3.39	62 20.54 x 3.39	11 29.01 x 3.36			+	+	+	43	White, appressed	Unchanged
3	9	4 –	10 21.84 x 3.46	4 –	75 38.92 x 3.26	7 –	+ Rare	+	+	51	White, appressed	do
4	8	Rare	Rare	Rare			+ Very abundant	+	+	51	White	do
5	2		Rare	Rare	Rare		+	+	+	30	White, appressed	do
6	6	17 17.28 x 3.67	55 17.28 x 3.39	6 –	22 31.30 x 3.33		+	+	+	50	White	do
7	4		60 19.49 x 3.29	7 –	33 30.32 x 3.42	Rare	+	+ Very abundant	+	43	do	do
8	5	1 –	4 –	2 –	93 38.26 x 3.59		+	+	+	48	do	do
9	1	51 6.26 x 1.17	41 7.33 x 1.17	7 –	1 –		+	+	+	30	do	do

[a] Based on 100 spores.
[b] The upper figure represents a percentage; the lower figures the size.
[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

TABLE 14.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on 5 per cent potato dextrose agar under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa						Terminal	Intercalary		Mycelium	Substrate
		0	1	2	3	4						
1	3	3 –	5 –	9 –	83 31.3 x 4.01		+	+	+	30	White	Dark brown
2	7	Macroconidia, if any, rare					–	+	+	43	White, appressed	Unchanged
3	9	4 –	42 22.17 x 3.52	20 26.08 x 3.72	31 37.29 x 3.62	3 –	+	+	+	51	do	do
4	8	Rare	Rare				+ Very abundant	+	+	51	do	do
5	2	Macroconidia, if any, rare					+	–	–	30	do	do
6	6	Rare					+	+ Rare	+ Rare	50	White	do
7	4	Macroconidia, if any, rare					+	–	–	43	do	do
8	5	23 24.2 x 3.32	45 24.78 x 3.49	3 –	27 28.04 x 3.52		+	+ Rare	+ Rare	50	do	Very dark brown
9	1	99 7.38 x 1.22	1 –				+	–	–	30	do	Unchanged

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

TABLE 15.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction of nine physiologic forms of Fusarium lini grown on steamed rice under the same conditions*

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

logic forms of Fusarium lini grown on steamed rice under the same conditions

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Microconidia[c]	Chlamydospores[c]		Age of culture in days	Color	
		Number of septa					Terminal	Intercalary		Mycelium	Substrate
		0	1	2	3						
1	3	Macroconidia, if any, rare				–	+	+	30	White	Light yellow
2	7	3 – 78 24.12 x 3.36		5 – 14 30.97 x 3.42		+	+ Rare	+ Rare	34	do	Light pink
3	9		Rare	Rare		+	+	+	51	White, appressed	Light brown
4	8	Rare	Rare			+	+ Rare	+ Rare	51	do	do
5	2	Macroconidia, if any, rare				+	+	+	30	White	do
6	6	Macroconidia, if any, rare				+	–	–	50	do	White with trace of purple at line of diffusion
7	4	Macroconidia, if any, rare				+	+	+	43	Very light brown	Unchanged
8	5	Macroconidia, if any, rare				+	–	–	45	White	White with trace of purple at line of diffusion
9	1	81 5.59 x 1.21	19 5.91 x 1.23			+	–	–	29	do	Light pink

[a] Based on 100 spores.
[b] The upper figure represents a percentage; the lower figures the size.
[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

TABLE 16.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color[d] reaction of nine physiologic forms of Fusarium lini grown on steamed sweet clover stems under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]					Microconidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa						Terminal	Intercalary	
		0	1	2	3	4				
1	3	1 –	8 –	25 23.15 x 3.49	66 22.71 x 3.62		+	+	+	30
2	7	6 –	6 –	4 –	88 32.63 x 3.39		+	+	+	34
3	9	Rare	Rare				+	+	+	51
4	8	Macroconidia, if any, rare					–	+ Very abundant	+	51
5	2	45 20.21 x 3.39	46 18.26 x 3.52	4 –	5		+	+	+	30
6	6	4 –	64 18.62 x 3.39	4 –	28 27.78 x 3.52		+	+	+	50
7	4	3 –	15 19.23 x 3.39	10 23.15 x 3.69	72 29.01 x 3.91	Rare	+	+ Very abundant	+	43
8	5	Culture dried up								
9	1	12 8.56 x 1.16	68 7.18 x 1.18	17 8.83 x 1.21	3 –		+	+	+	29

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
– indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

TABLE 17.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different*

TABLE 17.—*The sporulation, variation in number of septa of macroconidia, the size of macroconidia in the different septation classes, and the color reaction[d] of nine physiologic forms of Fusarium lini grown on steamed alfalfa stems under the same conditions*

Physiologic form	Accession no.	Percentage[a] of macroconidia in septation classes, and average size in microns of 10 spores in each class[b]				Microconidia[c]	Chlamydospores[c]		Age of culture in days
		Number of septa							
		0	1	2	3		Terminal	Intercalary	
1	3	Macroconidia, if any, rare				−	+ Very abundant	+	30
2	7	Macroconidia, if any, rare				−	+	+	31
3	9	Rare	Rare	Rare		+	+ Rare	+ Rare	51
4	8	50[e] 17.93 x 3.33	22 21.84 x 3.29	2 −	26 28.09 x 3.33	+	−	−	50
5	2	54 26.21 x 3.39	31 29.9 x 3.39	9 −	6 −	+	+	+	30
6	6	Macroconidia, if any, rare				+	+	+	50
7	4	15 22.85 x 3.72	34 20.86 x 3.39	22 23.47 x 3.69	29 27.39 x 3.65	+	+	+	43
8	5	Rare	Rare			+	+	+	50
9	1	56 6.97 x 1.15	41 7.29 x 1.16	2 −	1 −	+	+	+	29

[a] Based on 100 spores.

[b] The upper figure represents a percentage; the lower figures the size.

[c] + indicates presence of microconidia or chlamydospores.
− indicates absence of microconidia or chlamydospores.

[d] Mycelium white throughout and color of substrate remained unchanged.

[e] Based on 50 spores.

TABLE 18.—*Comparative cultural characteristics of nine physiologic forms of Fusarium lini when grown on the same and different media under the same conditions*

Physiologic form	Accession no.	Medium	Mycelium								Color		Sporulation				Age of colonies in days				
			Plumose	Appressed	Cottony	Floccose	Woolly	Tufted	Silky	Colwebby	Mycelium	Substrate	Abundant	Medium	Sparse	Absent	4	6	8	10	12
																	Size of colonies in mm.				
1	3						+				White to pink	Light brown				+	9	15	22	29	36
2	7	Potato					+				do	do	+				14	19	25	31	37
3	9				+	+	+				do	do	+				16	21	27	34	31
4	8	glucose			+		+				do	do	+				15	20	26	33	40
5	2						+				do	do	+				16	21	26	33	39
6	6	agar	+		+						do	do	+				17	23	30	37	44
7	4				+						do	Brown	+				16	22	29	36	43
8	5					+	+			+	do	Light brown	+				17	24	31	38	45
9	1				+						White	do	+				13	16	21	26	31
1	3							+			White	Brown			+		5	7	8	9	10
2	7	Prune		+					+		do	Unchanged		+			12	17	22	29	36
3	9			+					+		do	do		+			16	22	29	36	43
4	8			+					+		do	do			+		14	19	25	32	39
5	2			+					+		do	do			+		14	19	25	32	34
6	6	agar		+					+		do	do	+				14	19	26	33	40
7	4			+					+		do	Brown		+			17	23	29	36	43
8	5			+					+		do	Unchanged		+			15	20	26	32	38
9	1			+					+		do	do			+		11	14	19	24	30
1	3			+					+		White	Unchanged			+		10	17	23	29	35
2	7			+					+		do	do		+			14	19	25	31	37
3	9	Corn		+					+		do	do		+			16	22	25	36	44
4	8			+					+		do	do		+			15	20	26	33	40
5	2	meal		+					+		do	do			+		14	19	25	32	39
6	6			+					+		do	do	+				15	20	26	32	38
7	4	agar		+					+		do	do	+				14	20	26	33	40
8	5			+					+		do	do	+				15	20	26	32	38
9	1			+					+		do	do			+		12	17	22	27	33

TABLE 19.—*Comparative cultural characteristics of nine physiologic forms of Fusarium lini when grown on slightly acid peptone agar under the same conditions*

Physiologic form	Acc. no.	Color		Zoning			Mycelium				Diameter of colonies in mm. at end of eight days
		Mycelium	Substrate	Distinct	Moderate	Faint	Woolly	Cottony	Tufted	Appressed at margin	
1	3	Pink	Reddish		+			+			42
2	7	do	Unchanged			+	+			+	65
3	9	do	Light yellow			+	+			+	65
4	8	do	Unchanged			+	+			+	62
5	2	Light yellow	Light yellow			+	+			+	65
6	6	Pink	do	+			+		+		73
7	4	do	do			+		+			70
8	5	do	do	+			+		+		72
9	1	White	Cream	+			+		+		50

Effect of Medium on Cultural Characteristics

Appel and Wollenweber (2), Sherbakoff (8), and others have used the cultural characteristics of micro-organisms on different media to distinguish species of *Fusarium* which could not be differentiated on a morphological basis.

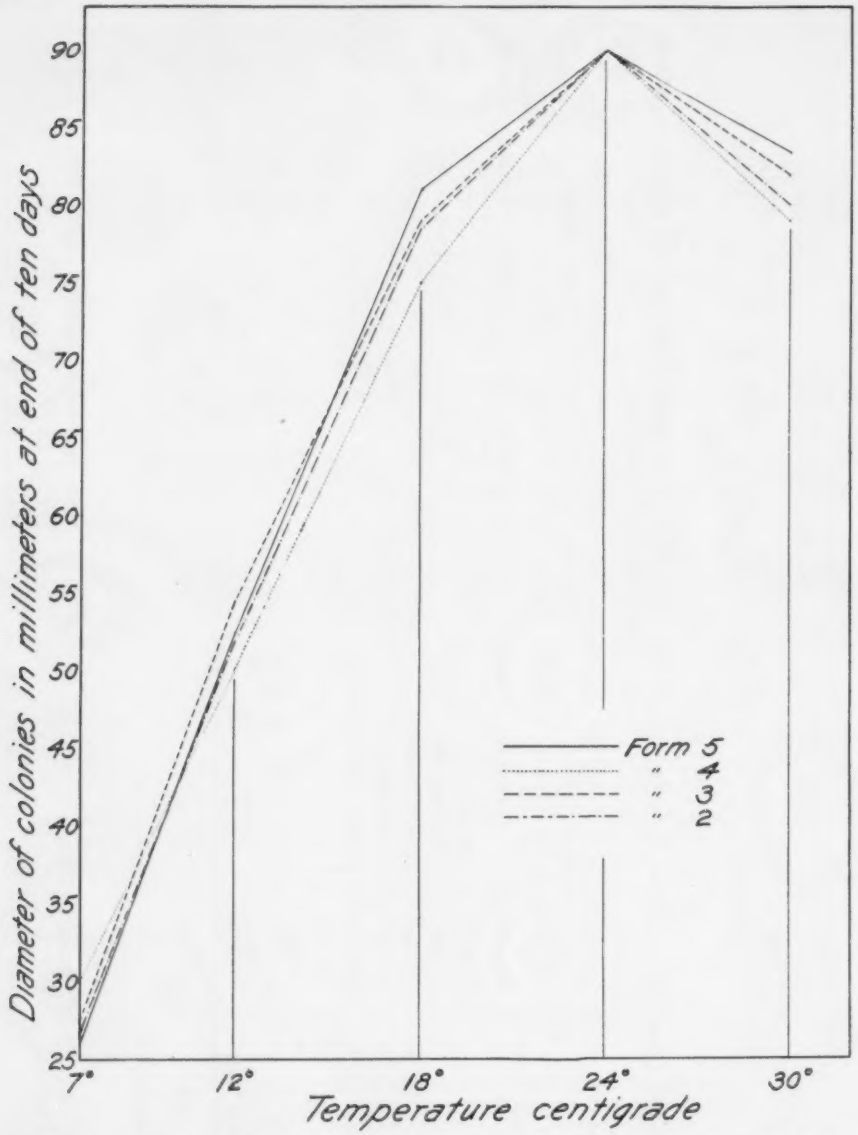

FIG. 3. Growth of four forms of *Fusarium lini* at the end of ten days on slightly acid peptone agar at various temperatures.

In order to compare their cultural characteristics, the nine forms of *F. lini* were grown on three different kinds of media: potato glucose agar, consisting of 20 grams of freshly peeled potato, 5 grams of sugar, 2 grams of agar, and 100 cc. of water; and synthetic cornmeal agar, and prune agar both manufactured by the Digestive Ferments Company, Detroit, Michigan. Triplicate plates of each medium were inoculated with small and, as nearly as possible, equal portions of mycelium of each form. Each medium was made in one lot, tubed, sterilized, and poured at the same time. All of the plates were kept under the same general environmental conditions. The nine forms listed in table 18 can be differentiated macroscopically, although with difficulty, by the following characters: color of mycelium and substrate, mycelial characters, sporulation, and rate of radial growth.

The organism which Bolley (5) described as a new species of *F. lini* was cultured on a slightly acid peptone agar. For this reason the cultural characteristics of nine forms of *F. lini* grown on slightly acid peptone agar are given in table 19.

Spore Germination

The process of spore germination in *F. lini* has been described by Bolley (5). The writer observed that one or more germ tubes, seldom more than two, are sent out from any portion of the spore, although usually from the ends. The size and number of germ tubes vary according to media and temperature.

TABLE 20.—*Summary of the percentage of germination of conidia of Fusarium lini, form 5, at various temperatures, in distilled water, with and without the addition of flax tissue, at the end of twelve hours*

Temperature in degrees C.	Flax tissue[a]	Percentage of germination				
		Trial				Average
		1	2	3	4	
7	–	16	12	10	12	12.50
	+	42	24	40	40	39.00
12	–	81	85	84	85	83.75
	+	83	90	86	90	87.25
18	–	86	84	84	82	84.00
	+	88	86	88	90	83.00
25	–	76	78	76	76	76.50
	+	82	90	86	90	87.00
30	–	71	73	74	90	90.00
	+	97	96	98	98	97.25
35	–	0	0	0	0	0
	+	0	0	0	0	0

[a] – indicates no flax tissue added; + indicates tissue added.

In distilled water at room temperatures, 20–21° C., the minimum time required for spore germination, that is, the first evidence of the germ tube protruding from the spore, was found to be 2 hours and 5 minutes in culture No. 14. When flax tissue was added to the medium, spores germinated in 1 hour and 45 minutes. The highest average percentage of germination in distilled water occurred over the rather wide range between 12° C. and 30° C. No spores germinated above 35° C., but some germinated at 7° C. At the end of 12 hours, at the lower temperatures, the germ tubes were not so long as they were at higher temperatures. Flax tissue, when added to the medium, stimulated germination greatly. These results are shown in table 20.

In tap water the spores germinated best at 30° C. In this medium also germination was stimulated when flax tissue was added (Table 21). From tables 20 and 21 it will be seen that germination was more profuse in the distilled water than in tap water.

TABLE 21.—*Summary of the percentage of germination of conidia of Fusarium lini, form 5, at various temperatures, in tap water with and without the addition of flax tissue, at the end of twelve hours*

Temperature in degrees C.	Flax tissue[a]	Percentage of germination				
		Trial				Average
		1	2	3	4	
7	–	8	6	8	6	7.00
	+	12	10	10	12	11.00
12	–	18	12	14	18	15.50
	+	70	18	16	18	18.00
18	–	18	26	30	26	25.00
	+	78	74	64	68	71.00
25	–	27	36	34	38	31.75
	+	56	74	78	60	67.00
30	–	88	90	90	78	86.50
	+	88	94	90	92	91.00
32.5	–	66	72	68	68	68.50
	+	78	74	76	72	75.00

[a] – indicates no flax tissue added; + indicates tissue added.

SUMMARY

1. Observational evidence seemed to indicate that physiologic specialization occurs in *F. lini*.

2. Experimental evidence proves that there are at least 9, and probably more, physiologic forms of *F. lini* which can be distinguished by their parasitism on the following four varieties of flax: Primost, Minn. 25 (C. I. 177);

Winona, Minn. 182 (C. I. 179); N. D. 3080 (C. I. 275); and N. D. 40013 (C. I. 241).

3. The four differential varieties vary in degree of resistance and susceptibility to seven physiologic forms.

4. Forms 1 and 2 are very virulent on all four differential hosts. Form 1, however, is quite distinct from form 2 and all other forms. When the plants inoculated with form 1 are 4–6 inches high, the green color gradually disappears from the tissues above the cotyledons until this portion of the plant is eventually colorless. The line of demarcation is very sharp between the normal and chlorotic tissues. This condition was produced by artificial inoculation in the field as well as in the greenhouse. Form 1 also causes loss of color in Manchuria barley, Victory oats, and Rosen rye. The green color gradually disappeared from the tissues above the first leaf.

5. The forms are numbered in order of their virulence, forms 1 and 2 being the most virulent, and forms 7, 8, and 9 the weakest.

6. Forms 2, 3, and 7 were found in the United States and Canada, while form 1 was found only in Saskatchewan, and the other forms in Minnesota. However, further collections might show the situation to be entirely different.

7. Variations in morphology of the spores of identical forms were found to exist under different conditions of growth. It was found necessary to measure at least 100 spores in order to obtain accurate results.

8. It was evident from this study that marked and significant differences occurred in spore size of a given form of *F. lini* when grown on several different media under the same environmental conditions. Therefore when studying the comparative morphology of forms of *F. lini* it is important to maintain uniform cultural conditions with respect to media and environment.

9. The mean length of 9 forms of *F. lini* grown on slightly acid peptone agar under uniform conditions is from $20.10 \pm .33$ microns for form 7 to $35.95 \pm .42$ microns for form 5. The mean width ranges from $3.77 \pm .03$ microns for form 3, to $4.08 \pm .04$ microns for form 5. Some of the differences are quite significant while others are not. Significant differences might have been obtained if thousands of spores had been measured. Therefore spore size of forms of *F. lini* is not a very accurate criterion for separating them into distinct morphological categories; but it indicates that there are inherent differences in the spore dimensions of different forms when cultured on the same medium under identical environmental conditions.

10. The taxonomic position of *F. lini* probably is in section Elegans of the form genus *Fusarium*.

11. No significant differences were found in the amount of radial growth of four forms of *F. lini* on the same medium at various temperatures.

12. The 9 forms can be distinguished with difficulty by macroscopic examination when grown on 3 different media under the same conditions.

13. The minimum time required for spore germination in distilled water at 20–21° C. was found to be 2 hours and 5 minutes for culture No. 14. Germination occurred in 1 hour and 45 minutes when flax tissue was added to the medium. Best germination in distilled water occurred between 12° C. and 30° C.; in tap water at 30° C. Flax tissue when added to the medium increased germination in all cases. No germination occurred above 35° C. Germ tubes of spores at 12° C. were shorter than those at higher temperatures. Some germination took place at 7° C. The percentage of germination was higher in distilled water than in tap water.

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LITERATURE CITED

1. ANDERSON, A. K. The biochemistry of *Fusarium lini*. Minn. Studies in Plant Sci., Studies in Biol. Sci. **5**: 1–43. 1924.
2. APPEL, O. and H. W. WOLLENWEBER. Grundlagen einer Monographie der Gattung *Fusarium* (Link). K. Biol. Anst. Land. und Forster. Arb. **8**: 1–207. 1910.
3. BABCOCK, E. B. and R. E. CLAUSEN. Genetics in relation to agriculture. 675 pp. McGraw-Hill Book Co. 1918.
4. BARKER, H. D. A study of wilt resistance in flax. Minn. Agr. Exp. Sta. Tech. Bul. 20: 1–42. 1923.
5. BOLLEY, H. L. Flax wilt and flax sick soil. N. D. Agr. Exp. Sta. Bul. 50: 1–57. 1901.
6. BROADFOOT, W. C. and E. C. STAKMAN. Physiologic specialization of *Fusarium lini* Bolley. Phytopath. **16**: 84. 1926.
7. CHRISTENSEN, J. J. The relation of soil temperature and soil moisture to the development of head smut of sorghum. Phytopath. **16**: 353–357. 1926.
8. SHERBAKOFF, C. D. Fusaria of potatoes. N. Y. (Cornell) Agr. Exp. Sta. Mem. 6: 1–269. 1915.
9. STOA, T. E. and A. C. DILLMAN. Flax seed production. N. D. Agr. Exp. Sta. Bul. 178: 1–43. 1924.
10. TISDALE, W. H. Relation of soil temperature to infection of flax by *Fusarium lini*. Phytopath. **6**: 412–413. 1916.
11. ————————. Flax wilt: a study of the nature and inheritance of wilt resistance. Jour. Agr. Res. **11**: 573–605. 1917.
12. WOLLENWEBER, H. W., C. D. SHERBAKOFF, O. A. REINKING, HELEN JOHANN, and A. A. BAILEY. Fundamentals for taxonomic studies on *Fusarium*. Jour. Agr. Res. **30**: 833–843. 1925.

PHYSIOLOGIC SPECIALIZATION AND MUTATION IN USTILAGO ZEAE[1]

J. J. Christensen and E. C. Stakman

INTRODUCTION

Corn smut, caused by *Ustilago zeae* (Beck.) Ung., apparently has been assuming increasing importance in corn production in the Upper Mississippi Valley. The disease may not be more destructive and prevalent now than it was 25 years ago; but, on account of the increase in corn production, the disease has attracted increasing attention during the past few years.

As was pointed out by Hayes et al (4), the best method of reducing losses from corn smut is the development of smut-resistant lines.[2] It is true

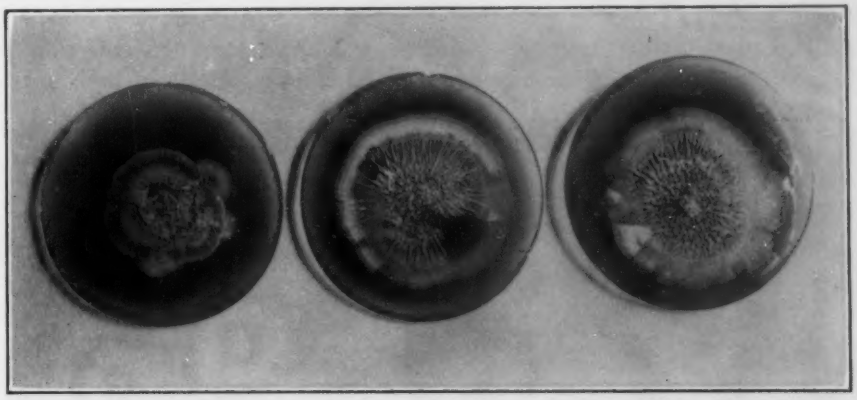

Fig. 1.—Three forms of *Ustilago zeae* showing sectors. Two or more distinct types of sectors appeared in each colony. From left to right: form 7 from Massachusetts; form 12 from New Hampshire; and form 1b from Minnesota, which itself was originally isolated from a sector.

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[2] For several years the Section of Plant Breeding and the Section of Plant Pathology of the University of Minnesota have cooperated on a project for the development of smut-resistant lines of corn. The investigation reported in this paper was made as a part of the general project.

that the destructiveness of the disease probably can be reduced somewhat by good cultural practices, sanitation, and proper soil fertilization. However, the only really adequate method of reducing losses would seem to be the use of resistant lines. Jones (5), Hayes et al (4) have demonstrated the possible feasibility of this method of controlling the smut. However, there is always the lingering possibility in developing resistant lines that they may remain resistant only as long as they are exposed to infection by the particular physiologic form or forms of the pathogene to which they were exposed during the process of their creation.

For several years there has been circumstantial evidence of the existence of physiologic forms of *U. zeae*. As a result of extensive observations, Melchers (6) pointed out, in 1920, the strong probability of the existence of forms of the pathogene. More recently, Stakman and Christensen (10) definitely demonstrated the existence of several forms. Tisdale and Johnston (12) also noted a difference in pathogenicity of different strains of the organism.

It seemed highly desirable, therefore, to make a thorough study of the number, characteristics, and geographic distribution of physiologic forms

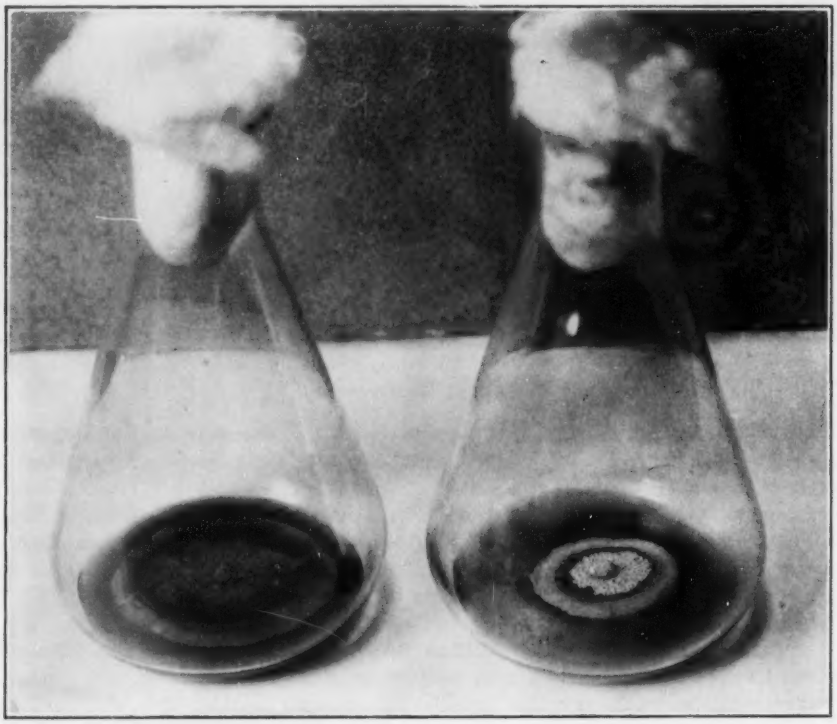

FIG. 2.—Mutant and its parent. Left, form 6a; right, parent form 6.

of *U. zeae* in the United States. It obviously is highly important also to ascertain whether these forms are relatively stable or whether they change rapidly. For the past two years, therefore, investigations have been made on these phases of the problem at the University of Minnesota.

MATERIALS AND METHODS

Twelve collections of *U. zeae* were obtained in 1924 and 1925, from the sources indicated in table 1. Cultures were obtained as follows: spores were

TABLE 1.—*Source of 15 physiologic forms of Ustilago zeae*

Form	Origin	Locality	Year	Collector
1	Smut boil	Minnesota (Isanti)	1925	Writers
1b	Sector from form 1	.....	1925	.....
2	Smut boil	Minnesota (University Farm)	1924	Writers
3	do	Manitoba, Canada	1924	D. L. Bailey
4	do	Nebraska	1925	A. F. Thiel
5	do	Ohio	1925	H. A. Rodenhiser
6	do	Texas	1925	W. Butler
6a	Sector from form 1	.....	1926	.....
7	Smut boil	Massachusetts	1925	H. A. Rodenhiser
8	do	Pennsylvania	1924	P. Brierley
8a	Sector from form 8	.....	1925	.....
9	Smut boil	Colorado	1925	A. E. Lungren
10	do	Mississippi	1924	J. M. Wallace
11	do	Maine	1925	D. Folsom
12	do	New Hampshire	1925	H. A. Rodenhiser

taken from a smut ball, from the inside whenever possible, and kept for 24–72 hours in a 1 per cent solution of copper sulphate, which was shaken from time to time in order to insure wetting of the spores. Some of the spore suspension was then removed from the container by means of a sterile platinum loop, and streak inoculations were made on a 2 per cent potato dextrose agar in petri dishes. Pure cultures often developed from this first inoculation. Transfers then were made, and stock cultures were kept at room temperature in the laboratory.

In order to compare the cultural characters of forms obtained from the different collections, transfers were made to several different media in triplicate petri dishes, flasks, or tubes. For these comparative studies, the agar always was made up in one lot, tubed, sterilized, and poured at about the

same time. After inoculation, the containers were kept at room temperature on the same table in the laboratory, in order that all might be subjected to the same general environmental conditions.

In order to obtain sporidia for inoculation, transfers were made from the different cultures to a 1 per cent carrot-dextrose decoction. Sporidia were produced in great abundance in this nutrient medium. Just before

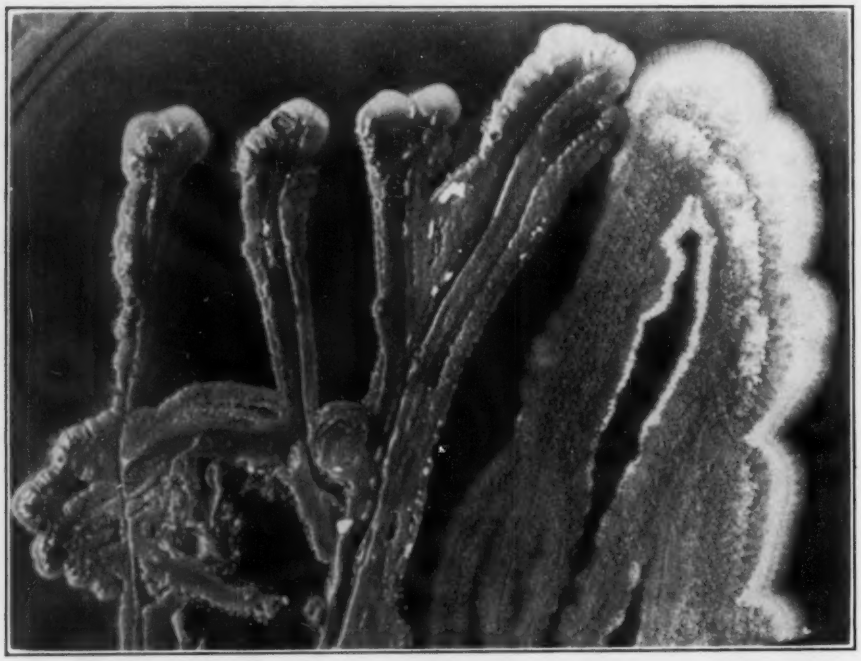

FIG. 3.—Two physiologic forms of *Ustilago zeae* growing in the same petri dish. On the left, form 6 from Texas; on the right, form 1 from Isanti, Minnesota.

the material was used for inoculating plants, an equal amount of fresh nutrient was added to each of the flasks.

Inoculations were made on selfed lines of corn[3] in the greenhouse and in the field. Two methods were used in the greenhouse: (a) numerous punctures were made with a sterile needle in the stalk of the corn plants, which were then sprinkled with a suspension of conidia; (b) about 1 to 1½ cc. of the suspension of sporidia was injected, with a hypodermic needle, into each stalk. Only the latter method was used in the field. Control plants, of course, always were punctured or injected with the sterile nutrient solution.

[3] All selfed lines of corn were furnished by the Section of Plant Breeding of the University of Minnesota. A selfed line of teosinte was obtained from the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

RESULTS

Cultural Characters of Different Forms.—Fifteen distinct forms were obtained, either directly from the original collections or as mutants from certain forms. There are striking and consistent cultural differences between the different forms. They easily can be differentiated on culture media by the following characters: rate of growth, color, relative amount of aerial mycelium, nature of mycelial growth, topography, zonation, surface, character of the margin, and abundance of conidia. The data on cultural characters are summarized in table 2.

The cultural characters of a given form are remarkably constant on the same medium and under the same general environmental conditions. However, they may differ greatly on different media and under different conditions of light, temperature, etc. Different forms sometimes look almost exactly alike on one medium but are entirely different in appearance on another. It should be emphasized, therefore, that cultural differences between forms can be detected only if the proper differential materials are used. And it may be worth while emphasizing the fact that there is a great deal of variation in the cultural characters of the same form under different conditions. Similar facts have been observed for various smut fungi by Sartoris (9), and by Christensen (1 and 2) for *Helminthosporium sativum* P. K. and B. The differences in cultural characters of different forms of *U. zeae* are sometimes far greater than those between the so-called species, *Ustilago tritici* (Pers.) Rost. and *U. nuda* (Jens.) K. and S., which, as Rodenhiser (8) recently has shown, are probably themselves only physiologic forms, and both of which, if one does consider them as separate species, contain many culturally distinct forms. Furthermore, some forms of *U. zeae* differ from each other more than they differ from certain forms of *U. nuda* and *U. tritici*. The writers also have observed such cultural differences between different collections of *Sorosporium reilianum* (Kühn) McAlpine.

There can be no question whatever regarding the distinctness of different physiologic forms in culture. (See Figs. 1–3.) Naturally, however, the most important question is, are these forms pathogenically distinct? And particularly important is the question as to whether any of them are so virulent as to be able to infect normally lines of corn which have been resistant in field experiments. Experiments were made to answer these questions.

For the field tests of comparative virulence of forms, ten lines of corn, which had been selfed from five to seven years, were selected. These lines had been grown for several years in the experimental plots at University Farm, St. Paul, where they were inoculated artificially each year. Their reaction to the form or forms of *U. zeae* at University Farm was therefore

TABLE 2.—*Cultural characters of 12 forms of Ustilago zeae grown under identical conditions on potato dextrose agar in Erlenmeyer flasks*

Form	Age in days	Diameter of colonies in mm.	Average radial growth per day	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
1	12	36.0	3.0	Deep tan to brown, grading to cream at margin	Umbonate, wrinkled and ridged	Velvety	++[a]	None	Filamentous, slightly undulate
	20	54.6	2.7	Deep purple to brown	do	do	++	None	do
1b	12	25.0	2.0	Cream to light tan	Convex to umbonate; wrinkled and radially ridged	Dull china-like to velvety	+	None	do
	20	39.1	2.0	Cream to light tan; white margin	Same, but more pronounced	Dull china-like to velvety, becoming powdery	+	None	do
2	12	31.0	2.5	Whitish, grading to yellow brown	Hummocky in center to striate on ray portion	China-like to powdery	+–	None	do
	20	42.0	2.1	Purplish brown	Sharply radially ridged, except in center and outside ray	Powdery to velvety	+	Faint	do
3	12	34.2	2.8	Center brown; margin white; remainder tan	Plane, convex to umbonate	Powdery to felt-like	++	None	do
	20	55.0	2.8	Center grayish, powdery	do	do	++	Moderate	do
6	12	24.0	2.0	Center gray-brown; margin yellowish white	Convex, umbonate; few shallow reticulations	Center powdery; margin china-like	0	Sharp near the edge	Not filamentous, undulate

Form	Age in days	Diameter of colonies in mm.	Average radial growth per day	Color	Topography	Surface	Amount of	Zonation	Margin

Form	Age in days	Diameter of colonies in mm.	Average radial growth per day	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
6	20	29.6	1.5	Outer part brownish	Convex, umbonate; few shallow reticulations	Center powdery; margin china-like	0	Sharp near the edge	Not filamentous, undulate
6a	12	34.3	2.9	Purple-brown	Verruculose in center, grading to a plane surface	Velvety	++	None	Filamentous, slightly undulate
	20	53.0	2.6	Center gray, grading to dark purple	Rugose, wrinkled, ridged, except ray portion	Powdery to velvety	++	None	do
7	12	25.6	2.1	Cream to brown, scattered white patches	Raised to umbonate, slightly wrinkled	China to powdery	+=	Moderate (Margin only)	Slightly wavy to lobate
	20	32.3	1.6	do	An increase in wrinkles; sectors present	do	+=	do	do
8	12	25.6	2.1	White to tan	Convex, plane; rugose in spots	China-like	+=	Faint	Undulating in parts, myceloid
	20	38.5	1.9	do	Convex, plane; rugose in spots, sectors present	do	+=	do	do
8a	12	35.5	2.9	Center tan, rays white	Umbonate and appressed, border plane	Velvety to felty	++	None	Myceloid, slightly undulate
	20	50.5	2.5	do	do	Velvety	++	Moderate	do
10	12	41.3	3.4	Light purple	Reticulated, convex to umbonate	Center dull, edge waxy	0	None	Deeply lobed
	20	54.5	2.7	Light purple, grayish margin	Reticulated, convex to umbonate, appressed margin	Edge velvety	+ (Margin only)	Prominent (Margin only)	Deeply lobed, myceloid

TABLE 2.—(*Continued*)

Form	Age in days	Diameter of colonies in mm.	Average radial growth per day	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
11	12	35.3	2.9	Gray-brown	Convex, plane, slightly wrinkled in patches	Powdery	++	None	Filamentous, undulate
	20	56.3	2.8	Gray-purple	do	do	++	Slight	do
12	12	32.3	2.6	Whitish-yellow to brown	Convex, papillate in center	China-like to powdery	+−	None	do
	20	49.0	2.5	Center cream to lilac, margin white	Convex, papillate in center; sectors present	Velvety	+−	Slight	do

[a] ++, abundant; +, moderate; +−, slight; +=, trace; 0, absent.

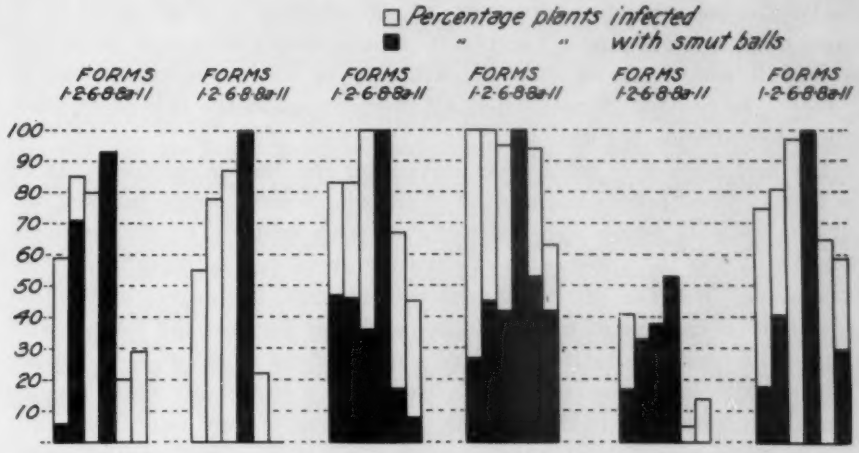

FIG. 4.—The reaction of selfed lines of corn to six physiologic forms of *Ustilago zeae*. Strains of corn, left to right, are: Longfellow (Culture 700), Longfellow (Culture 6000), Rustler (Culture 4400), Rustler (Culture 3300), N. W. Dent (Culture 4100), Brown Aleurone (Culture 1700).

known. Resistant (low smut), susceptible (high smut), and intermediate lines were selected.

One long row of each line was planted early in June. The rows were 3½ feet apart and the hills were 1 foot apart in the rows. The plot then

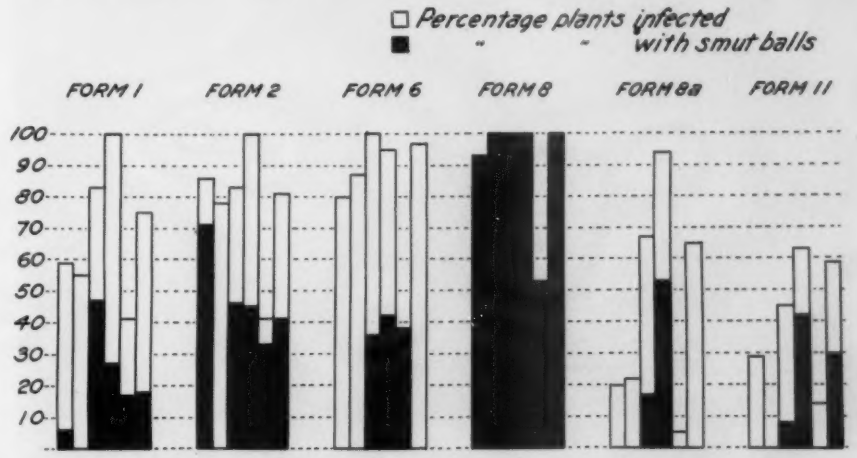

FIG. 5.—The reaction of selfed lines of corn to six forms of *Ustilago zeae*. For each physiologic form, the six lines of corn, from left to right, are: Longfellow (Culture 700), Longfellow (Culture 6000), Rustler (Culture 4400), Rustler (Culture 3300), N. W. Dent (Culture 4100), Brown Aleurone (Culture 1700).

was subdivided into 12 subdivisions, each containing from about 20 to 30 plants of each selfed line. The plants in each subdivision were inoculated on July 9, and again on July 24, with one of the physiologic forms of *U. zeae*. As previously mentioned, all inoculations in the field were made with a hypodermic syringe.

The first notes were taken seven days after the first inoculation. All of the forms caused infection and most of the plants had become infected, except the controls, which were entirely free from smut. The first evidence of infection was the appearance of chlorotic areas, two or three days after inoculation. Necrosis often followed the chlorosis, the degree and extent depending on the line of corn and the particular form of the fungus with which it had been inoculated. There were no signs of chlorosis nor other evidences of infection on the controls, except on a few plants which became naturally infected late in the summer. By August 9 there were large smut boils on many plants, while others apparently had outgrown the infection.

There were striking differences, corresponding with those observed in the greenhouse, in the pathogenicity of different forms (Table 3 and Figs.

FIG. 6.—Left: Salmon Silk (Culture 1800—1925); only very small smut boils developed. Right: Brown Aleurone (Culture 1700—1925): note large smut boils and distortion of plants. Of 21 plants inoculated in field, 20 were killed.

4 and 5). At least 7, and probably 8, of the 12 forms with which inoculations were made were decidedly different. Some of them differed from each other in their effect on all 10 of the lines of corn, while others differed only in their action on a few of them.

The differences in the effect of the different forms sometimes were very sharp. Certain forms produced only very minute smut boils on some lines of corn; others produced very large smut boils on nearly all lines; and still others characteristically caused very sharp chlorosis on many lines. In fact, there were remarkably sharp differences in the effect of the different forms. Some were extremely virulent, others moderately so, and still others only weakly pathogenic. (See Figs. 6–8.)

Form 8, from Pennsylvania, was the most virulent of all. This form infected nine of the ten selfed lines of corn heavily, and produced large smut boils on practically all plants in each plot. It not only caused heavy infection on susceptible lines of corn but also on some which had been resistant at University Farm for several years. For instance, it killed outright 20 of the 21 inoculated plants of Rustler, culture 4400, a "resistant line." The only line of corn resistant to this form was Salmon Silk, culture 1800. (See Figs. 6, 9, 10.)

Form 6, from Texas, easily could be distinguished from all the other

FIG. 7.—The effect of two physiologic forms of *Ustilago zeae* on a "resistant" line of corn, Minnesota 13 (Culture 4000—1925), selfed five years. On the left, form 8a obtained from a sector from form 8 on the right.

forms because of the extensive and pronounced chlorosis which is caused on several of the lines of corn.

Form 7, from Massachusetts, was the weakest. Only one line of corn, Rustler, culture 3300, was susceptible to it. On all other varieties the only indication of infection was a slight chlorosis and, occasionally, small smut boils.

It is quite obvious from the results that many of the lines of corn which have been highly resistant in the regular field tests of previous years were completely susceptible to some of the forms of the smut fungus. On the other hand, some of the susceptible lines of corn were resistant to the weakest of the forms. It is quite clear from the results that one is not justified in speaking of resistant or susceptible lines of corn unless one specifies the particular forms of the pathogene involved. For instance, Longfellow, culture 6000, was resistant to 11 of the 12 forms, but it was quite susceptible to form 8.

Stability of the Physiologic Forms.—The range of variability of forms of *U. zeae* is very wide. The character and the rate of growth, the ability to produce sporidia, and the morphology of the organism are influenced profoundly by the kind, proportion, and amount of food available, and by other

FIG. 8.—The effect of two physiologic forms of *Ustilago zeae* on a "resistant" line of corn, Rustler (Culture 4400—1925), selfed six years. On the left, form 8; right, form 7.

Form	King Phillip Culture 6200 Susceptible[a]						Longfellow Culture 6000 Moderately resistant						Longfellow Culture 700 Resistant						Rustler Culture 3300 Susceptible						Rustler Culture 5800 Moderately susceptible					
	No. plants inoculated	Percentage infected	Necrosis[b]	Chlorosis[b]	No. plants with boils	Abundance and size of boils[b]	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils
1	15	100	n	c	3	1 f	18	55	n =	c =	0	–	17	59	n =	c =	1	2 f –	15	100	n –	c +	4	1 to 5 f	19	83	n –	c –	2	2 f
1b	20	95	n +	c +	3	1 = f	18	6	n =	c =	0	–	15	93	n –	c =	0	–	18	83	n –	c	4	1 to 4 f	20	90	n –	c –	1	4 f
2	18	100	n	c	18	1=to 1+ a	18	78	n –	c	0	–	14	86	n =	c –	10	3 m	20	100	n –	c +	9	1 to 4 a	18	100	n =	c	6	1 to 4 f
3	14	72	n =	c –	2	1 – f	19	16	n	c	1	1 f	14	78	n –	c –	0	–	16	93	n =	c +	7	1 to 4 f	29	72	n =	c =	3	3 f
6	13	100	n –	c	1	1 = f	16	87	n =	c =	0	–	15	80	n =	c	0	–	19	95	n –	c ++	8	1 to 3 f	20	85	n –	c –	3	1 to 3 f
6a	18	83	n –	c –	1	1 = f	18	33	n =	c =	0	–	14	29	n =	c =	0	–	18	61	n –	c ++	3	1 to 4 f	20	75	n =	c =	4	1 to 3 f
7	19	0	–	–	–	–	12	0	–	–	–	–	17	0	–	–	–	–	14	64	n =	c =	9	1 to 5 m	18	17	n =	c =	0	–
8	16	100	n –	c +	16	2 to 4 a	18	100	n =	c –	18	2 to 3 a	15	93	n =	c =	14	1 to 5 a	16	100	n –	c –	16	1 to 5 a	23	100	n –	c	23	2 to 5 a
8a	15	40	n =	c =	2	1 = f	18	22	n =	c =	0	–	15	20	n =	c =	0	–	17	94	n =	c	9	1 to 4 f	29	74	n –	c –	2	2 – f
10	10	30	n =	c =	2	1 to 2 f	20	45	n =	c –	1	1 = f	10	60	n =	c =	0	–	18	78	n =	c =	9	1 to 4 m	27	24	n =	c =	0	–
11	16	75	n =	c –	2	1 = f	17	0	–	–	–	–	14	29	n =	c =	0	–	19	63	n –	c –	8	1 to 4 f	18	55	n =	c =	4	1 to 5 f
12	7	100	n	c	2	1 = f	33	13	n =	c =	0	–	6	50	n =	c =	0	–	17	94	n =	c –	3	1 to 2 f	18	67	n –	c –	0	–
Control	20	0	–	–	–	–	17	6	0	0	0	–	15	0	0	0	0	–	15	40	n =	c =	6	1 to 4 f	20	15	n[0]	c[0]	3	2 to 4 f

TABLE 3.—(*Continued*)

Form	Rustler Culture 4400 Resistant						Minn. No. 13 Culture 4000 Resistant						N. W. Dent Culture 4100 Resistant						Brown Aleurone Culture 1700						Salmon Silk Culture 1800					
	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils	No. plants inoculated	Percentage infected	Necrosis	Chlorosis	No. plants with boils	Abundance and size of boils
1	30	83	n =	e	14	1to5 a	17	100	n =	e =	0	–	29	41	n –	e =	5	1to5 f	28	75	n =	e –	5	1to4 f	19	43	n =	e =	0	–
1b	29	97	n –	e	3	1to3 f	15	87	n =	e –	0	–	29	100	n –	e++	0	–	29	90	n	e	10	1to5 m	18	28	n =	e =	0	–
2	24	83	n –	e –	11	1to3 a	14	93	n =	e –	9	1to2 f	30	33	n =	e =	10	1to2 f+	27	81	n =	e –	11	1to4 m	21	62	n –	e	0	–
3	18	67	n =	e =	0	–	26	81	n =	e –	0	–	19	0	–	–	–	–	17	41	n =	e =	2	1to3 f	24	41	n –	e –	0	–
6	28	100	n =	e++	10	1to3 f	16	100	n –	e++	2	1to3 f	29	38	n =	e =	11	1to3 m	28	97	n =	e+	0	–	17	88	n =	e	0	–
6a	26	65	n	e –	3	1to2 f	16	93	n =	e =	0	–	27	22	n =	e =	0	–	29	75	n =	e –	7	1to2 f	19	42	n –	e =	0	–
7	29	50	n =	e =	2	1to3 f	13	46	n =	e =	0	–	29	0	–	–	–	–	27	26	n =	e =	6	1to5 f	19	37	n =	e =	0	–
8	21	100	n =	e	21	1to5 a	15	100	n =	e –	15	1to5 a	19	53	n =	e =	10	1to4 m	21	100	n =	e –	21	1to5 a	15	80	n =	e –	9	1–to2e a
8a	18	67	n =	e –	3	1to2 f	20	100	n =	e	3	1to2 f	18	5	n =	e =	0	–	17	65	n =	e	3	1to4 f –	22	69	n =	e =	0	–
10	11	72	n =	e =	0	–	27	85	n =	e =	1	1 f	10	0	–	–	–	–	19	15	n =	e =	2	1to3 f –	27	37	n =	e =	0	–
11	24	45	n =	e =	2	1to4 f –	17	29	n =	e =	1	1+ f	29	14	n =	e =	0	–	27	59	n =	e =	8	1to5 f+	19	32	n =	e =	0	–
12	10	70	n =	e =	0	–	23	–	–	–	–	–	10	–	–	–	–	–	30	–	–	–	–	–	20	–	–	–	–	–
Control	27	4	0	0	1	1 = f =	15	0	–	–	–	–	29	0	–	–	–	–	27	3	0	e =	3	1to4 f	20	0	–	–	–	–

environmental conditions. Comparative cultural tests made during the past two years indicate that some physiologic forms remain constant and always appear the same under identical conditions. Temporary phenotypes, or modifications, induced by differences in environmental conditions, occur frequently, although the genotype remains unaltered. Such variants revert to the parental phenotype as soon as the causal stimulus is removed.

However, some forms mutated frequently, if one can designate as mutants the wedge-shaped sectors which often occur in cultures. There was mutation in forms obtained from eight different states. Of all forms studied, only four failed to produce any mutants. Some of the forms mutated much more readily than others. Form 12, for instance, produced at least three distinct mutants in the same culture.

That these so-called mutants really are new genotypes, and not mere phenotypes caused by environmental conditions, is indicated by the fact that they bred true during repeated subsequent transfers. Some of these mutants have been grown on many different kinds of media, and under different conditions, for a considerable period of time without losing their distinctive character. Form 8a has been cultured for 1½ years and form 6a for about 10 months, and during this time they have remained constant.

Some of the mutants again mutated. Thus, from a single isolation made from a smut boil collected at Isanti, Minnesota, six distinct mutants were obtained, all of which retained their distinctive characters after repeated transfers (Fig. 11). Form 1bba (Fig. 11) was obtained through a series of three successive and distinct sectors, and a culture of this form produced three other distinct sectors. It is possible that the last three may be variants rather than mutants, as they have not been studied very long. It may be noteworthy that the last sector obtained from this series of cul-

TABLE 3.—(*Continued*)

[a] The terms "susceptible," "moderately resistant," and "resistant" indicate the reaction of the selfed lines of corn to smut under field conditions at University Farm when inoculated artificially with *U. zeae* from University Farm.

[b] Explanation of symbols:

n and c denote the degree of necrosis and chlorosis, respectively.

a indicates an abundance of smut boils.

m indicates a moderate number of smut boils.

f indicates few boils.

A plus or minus sign further differentiates the class within the respective groups.

The numerical values 0 to 5 indicate the relative size of the smut boils. 0 indicates absence of boils; 1 indicates boils varying from warts to ½ inch; 2 = ½ to 1 inch; 3 = 1 to 2 inches; 4 = 2 inches to 3 inches; 5 = 3 inches or above.

[c] Mostly 1 infections.

tures, *i.e.*, 1bba, differs much more from its parent, form 1, than 1a or 1b which developed earlier in the series of cultures.

The possibility is not excluded that these sectors which have been designated as mutants may arise from normal segregation. Sartoris (9) states that sporidia of *U. zeae*, when placed on glycerine agar, germinate and produce germ tubes which conjugate. A binucleate mycelium is thus formed. However, the shape of the sectors, and their mode of origin, suggest mutation rather than segregation. The phenomenon is similar to what has been interpreted as mutation in many other fungi. A fuller study is necessary, however, before a definite statement can be made. But, from the pathological standpoint, the essential fact is that sudden and permanent changes occur in physiologic forms of *U. zeae*. The most important question naturally is: is there a change in pathogenicity corresponding with a change in appearance?

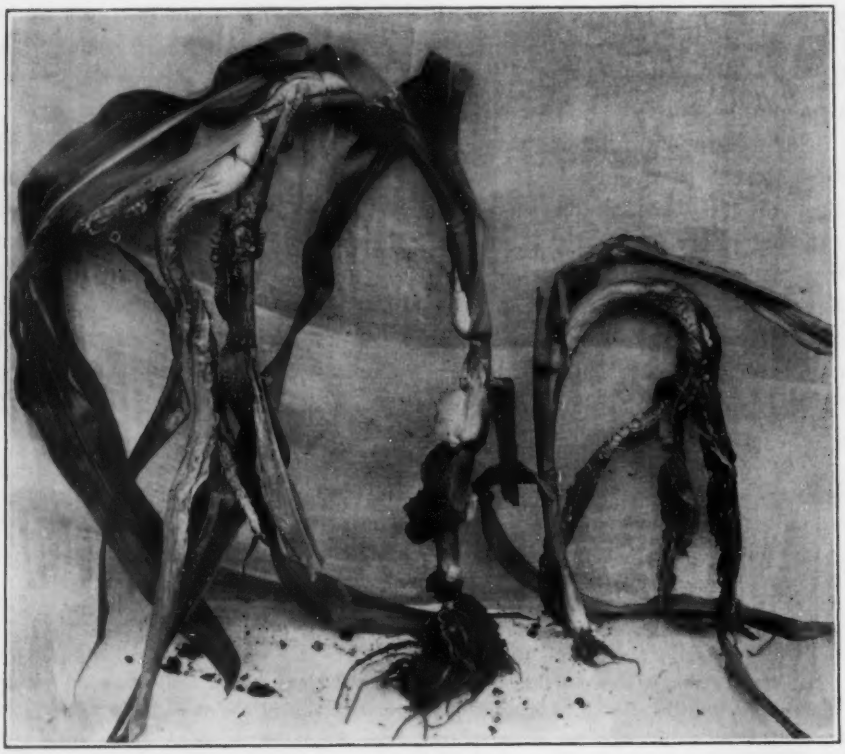

FIG. 9.—A "resistant" line of corn, Rustler (Culture 4400—1925), inoculated with form 8, from Pennsylvania. This was the typical appearance in the plot of all the plants of the line.

Pathogenicity of Mutants.—In order to ascertain whether mutants were pathogenically different from their parents, three of them were included in the field experiments already described. Mutants 8a, 6a, and 1b were used. All ten lines of corn were inoculated with them. Mutant 1b was approximately as virulent as its parent; 6a was slightly less virulent than its parent, while 8a, which was obtained from the most virulent of all forms studied, was decidedly less virulent than its parent. In fact, the latter mutant seemed to have lost its pathogenicity almost completely. There was a tremendous difference between the pathogenicity of its parent and that which it itself showed. (See table 3 and fig. 7.)

There is further evidence on the constancy of mutants from the inoculation experiments. Form 8 and its mutant 8a have been transferred approximately the same number of times in culture and have been grown under many different environmental conditions, yet after 21 months their cultural characters are still distinct, and their pathogenic capabilities remained the same after at least six months in culture.

The loss in virulence indicated above can not be attributed to attenuation in culture, because forms 2 and 8 are very virulent, while forms 7 and

FIG. 10.—Two selfed lines of corn severely injured by a virulent form of *Ustilago zeae*, form 8, from Pennsylvania. Front row, Longfellow (Culture 700—1924). Back row, Rustler (Culture 3700—1925). Both strains had previously proved resistant to *U. zeae* for several years at University Farm.

12 are weakly pathogenic, although the first two have been cultured at least a year longer than the two latter.

The fact may or may not be significant that none of the mutants tested were more virulent than their parents. However, Christensen (2) has shown that mutants of *Helminthosporium sativum* sometimes are more virulent than their parents, and it is entirely possible that the same thing may prove to be true of *U. zeae*.

DISCUSSION AND CONCLUSIONS

There are many physiologic forms of *U. zeae* which differ greatly in cultural characteristics as well as in pathogenicity. It is unfortunate but true that many supposedly resistant lines of corn probably are resistant in restricted localities and for limited periods of time only. This is clearly shown because of the fact that selfed lines of corn which had been highly resistant for several years at University Farm were completely susceptible to forms obtained from other states. It also is significant that several different forms of *U. zeae* may occur within a rather restricted geographical locality. For instance, one form was obtained at University Farm, St. Paul, and another rather distinct one was collected approximately 50 miles away.

The fact that forms mutate makes it rather difficult to be sure of the situation in breeding resistant lines, because the situation is constantly

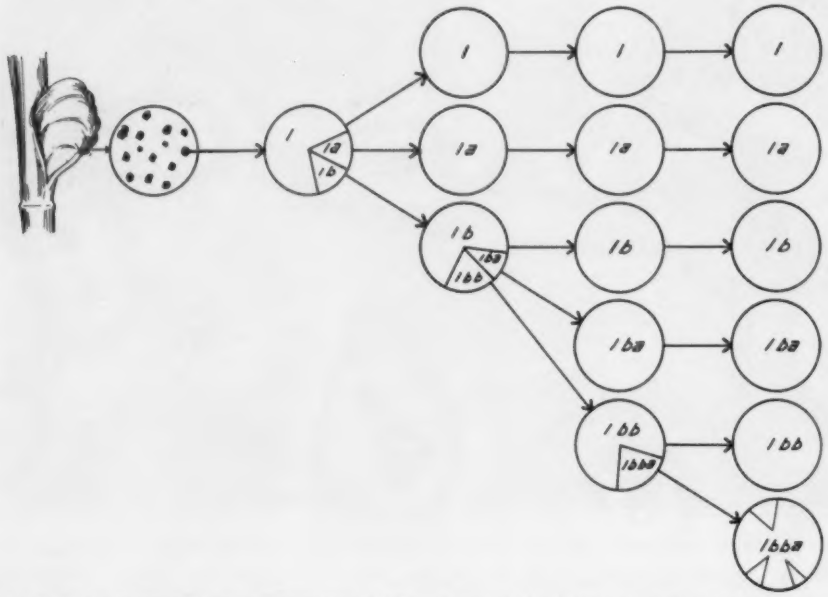

FIG. 11.—Diagrammatic representation of the origin of five distinct variants through successive sectoring in culture media from a single isolation of *Ustilago zeae*.

changing. It is to be hoped that the mutants of *U. zeae* are always less virulent than their parents. But there is no assurance and little hope, from what is known of other fungi, that this is true. It seems likely that breeding for smut resistance must be done for certain localities, and it is likely that it may have to be done more or less continuously. In view of the fact that there are many and changing physiologic forms of the pathogene, the corn smut problem probably can not be solved easily, once and for all. It probably will be a contest between a pathogene which is continually shifting its base, and breeders who are trying to keep a few steps ahead of the pathogene. How many physiologic forms there are, what their distribution is, and exactly how much they will complicate the breeding problem remains to be seen. The writers make no predictions. This much, however, is certain: there are physiologic forms of *U. zeae*, and lines of corn which have been resistant for several years to certain physiologic forms are completely susceptible to others. A line may be resistant in one region and completely susceptible in another; and it may be relatively free from smut in one locality for some time, and then become heavily infected on account of the advent of a form of smut to which it is susceptible.

Whatever the ultimate importance of physiologic specialization may prove to be, it is essential to take the phenomenon into consideration in breeding work. Information must be obtained regarding the number of physiologic forms, their geographic distribution, their degree of constancy, their pathogenic capabilities, and the degree of mobility.

The question naturally arises as to how rapidly a virulent form may be disseminated if it occurs only in a restricted locality. Theoretically it should be possible for the fungus to spread very rapidly. Almost inconceivably large numbers of sporidia are formed; they retain their viability under adverse conditions for months, as Piemeisel (7) has shown; and they have been caught several thousand feet high in the air (11).

While the problem of developing resistant lines of corn is complicated by the existence of physiologic forms, breeding work will still be valuable and may eventually solve the corn smut problem. However, the methods of developing the resistant lines must be changed. It will be necessary to inoculate them with all of the forms which occur in a region for which the varieties are intended, and it may be necessary to resort to more crossing and recrossing in order to develop resistant lines synthetically, as is now being done in developing varieties of wheat resistant to stem rust (3).

SUMMARY

1. *Ustilago zeae* is a group species comprising numerous physiologic forms.

2. The writers studied fifteen forms in considerable detail. The forms can be distinguished in culture by the following characters: rate of growth, color, topography, surface, zonation, conidial production, and margin.

3. At least seven, and possibly eight, forms can be recognized by their parasitic behavior on ten selfed lines of corn.

4. Some forms are very virulent, some moderately so, while some are relatively innocuous. The most virulent form was obtained from Pennsylvania, and the weakest one from Massachusetts.

5. Some selfed lines of corn which had been resistant in the field for several years at University Farm, St. Paul, were completely susceptible to at least one of the physiologic forms.

6. Mutants occur frequently on culture media as wedge- or fan-shaped sectors. Transfers from these sectors continued to be distinct from their parents when propagated on culture media.

7. Some forms mutate readily, others do not. As a result of mutation, at least six distinct variants or mutants were obtained in a series of transfers from a single isolation. Three of these six variants originated from successive sectors.

8. The mutants differ from their parents not only in general appearance on culture media, but also in pathogenicity.

9. The mutants are as stable as forms isolated from chlamydospores obtained from smut boils. One mutant bred true for at least one and one-half years on culture media, while its pathogenic capabilities have remained constant for at least six months.

10. Distinct cultural differences were observed also between collections of *Sorosporium reilianum*.

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LITERATURE CITED

1. CHRISTENSEN, J. J. Studies on the parasitism of *Helminthosporium sativum*. Minn. Agr. Exp. Sta. Tech. Bul. 11. 1922.
2. ———. Physiologic specialization and mutation in *Helminthosporium sativum*. Phytopath. **15**: 785–795. 1925.
3. HAYES, H. K., and E. C. STAKMAN. Wheat stem rust from the standpoint of plant breeding. Read at annual meeting of Western Canadian Society of Agronomy held at Winnipeg, Manitoba, December 27–29, 1921.
4. ———, E. C. STAKMAN, FRED GRIFFEE, and J. J. CHRISTENSEN. Reaction of selfed lines of maize to *Ustilago zeae*. Phytopath. **14**: 268–280. 1924.
5. JONES, D. F. Selection in self-fertilized lines as the basis for corn improvement. Jour. Amer. Soc. Agron. **12**: 77–100. 1920.

6. MELCHERS, L. E. Ecologic and physiologic notes on corn smut, *Ustilago zeae* (Abst.). Phytopath. **11**: 32. 1921.
7. PIEMEISEL, F. J. Factors affecting the parasitism of *Ustilago zeae*. Phytopath. **7**: 294–307. 1917.
8. RODENHISER, H. A. Physiologic forms of *Ustilago nuda* and *Ustilago tritici*. Phytopath. **16**: 1001–1007. 1926.
9. SARTORIS, GEORGE B. Studies in the life history and physiology of certain smuts. Amer. Jour. Bot. **11**: 617–647. 1924.
10. STAKMAN, E. C., and J. J. CHRISTENSEN. Physiologic specialization of *Ustilago zeae* and *Puccinia sorghi* and their relation to corn improvement (Abst.). Phytopath. **16**: 84. 1926.
11. STAKMAN, E. C., A. W. HENRY, G. C. CURRAN, and W. N. CHRISTOPHER. Spores in the upper air. Jour. Agr. Res. **24**: 599–606. 1923.
12. TISDALE, W. H., and C. O. JOHNSTON. A study of smut resistance in corn seedlings grown in the greenhouse. Jour. Agr. Res. 32: 649–668. 1926.

PHYSIOLOGIC SPECIALIZATION OF USTILAGO NUDA AND USTILAGO TRITICI[1]

H. A. RODENHISER[2]

INTRODUCTION

Physiological specialization has long been known in the smut fungi, although the fact was not stated definitely. *Ustilago nuda* (Jens.) K. and S. and *U. tritici* (Pers.) Rost. really are physiologic forms, not good morphologic species. When *U. segetum* (Bull.) Ditt. was separated into several species, the basis for separation, at least as far as *U. nuda* and *U. tritici* are concerned, was pathogenicity, not morphology. Had the early investigators of the cereal smut fungi known what is now known about physiological specialization, it is likely that they would have considered *U. nuda* and *U. tritici* as physiologic forms of one morphologic species rather than as two morphological species. In fact, Jensen (4) did not at first elevate them to the rank of species, but considered them as varieties under the names *U. segetum* var. *tritici* and *U. segetum* var. *hordei nuda*. The morphological differences between these two fungi are scarcely pronounced enough to justify considering them as species, although it may be convenient to do so. The principal differences between them are physiologic rather than morphologic. They differ pathogenically as well as culturally, as Appel and Riehm (1) have shown.

It seemed quite probable, therefore, that there might be physiological specialization within the so-called species of loose smut fungi. This probability seemed the stronger in view of the fact that Reed (5) has demonstrated the existence of parasitic strains within *U. avenae* (Pers.) Jens. and *U. levis* (K. and S.) Magn., and Faris (2) within *U. hordei* (Pers.) K. and S., while Stakman and Christensen (7) and Tisdale and Johnston (8) have shown the same for *U. zeae* (Beck.) Ung. The writer therefore began experiments to ascertain whether there are similar strains or forms within *U. tritici* and *U. nuda*. As a preliminary to the investigation of the possible existence of pathogenic strains, an attempt was made to find out whether there were strains which could be recognized by differences in cultural characteristics on nutrient media.

[1] Published with the approval of the Director as Paper No. 645 of the Journal series of the Minnesota Agricultural Experiment Station.

[2] The writer wishes to acknowledge the helpful advice and criticism of Dr. E. C. Stakman, under whose direction the work was done.

MATERIALS AND METHODS

Collections of *U. nuda* and *U. tritici* were obtained during 1925 from widely separated localities as indicated in tables 1 and 2.[3] Pure cultures of the smuts were often obtained by transferring the chlamydospores directly to the culture medium. In other cases, chlamydospores were suspended in a one per cent solution of copper sulphate for from 10 to 27 hours. Pure cultures were then obtained by directly transferring the chlamydospores to the media by means of a platinum loop. A two per cent potato-dextrose agar proved to be the best medium for growth from which to study cultural differences of the physiologic forms of the two smuts. The pure cultures were grown for a period of two weeks on equal quantities of potato-dextrose agar. At the end of this time, triplicate series of 200 cc. Erlenmeyer flasks, containing 20 cc. of two per cent potato-dextrose agar, were inoculated with small equal portions of each culture. All of the cultures were subjected to the same environmental conditions. The agar was made up in one batch, poured and autoclaved at the same time; and the cultures were grown at room temperatures in the laboratory.

EXPERIMENTAL RESULTS

It is evident that there are distinct forms or strains, both of *U. tritici* and *U. nuda*. Cultures of *U. nuda* from six different sources and of *U. tritici* from three different sources were studied in detail. The forms can be differentiated on culture media by the following cultural characters: color, topography, character of surface, presence or absence of aerial mycelium, total amount of radial growth, and type of margin. The data on cultural characteristics are summarized in tables 1 and 2.

The cultural characteristics of six collections of *U. nuda* were distinctly different (Fig. 1). The same was true of the three cultures of *U. tritici* (Fig. 1). Furthermore, cultural differences in the various forms of *U. nuda* and *U. tritici* were as great as the cultural differences between the two species. There was, however, a distinct similarity between the culture of *U. nuda* obtained from material collected in Bulgaria and that of *U. tritici* from Egypt.

There were no apparent differences in the morphology of the various forms of the two smuts. Likewise, no microscopic differences were noted in the vegetative growths of cultures of *U. nuda* and *U. tritici* of the same age. All of the morphologic structures of *U. tritici* formed in culture and described by Sartoris (6) were noted. Chlamydospores of both *U. nuda* and *U. tritici* developed in culture.

[3] For spore material, the writer is indebted to the several people who are listed in tables 1 and 2.

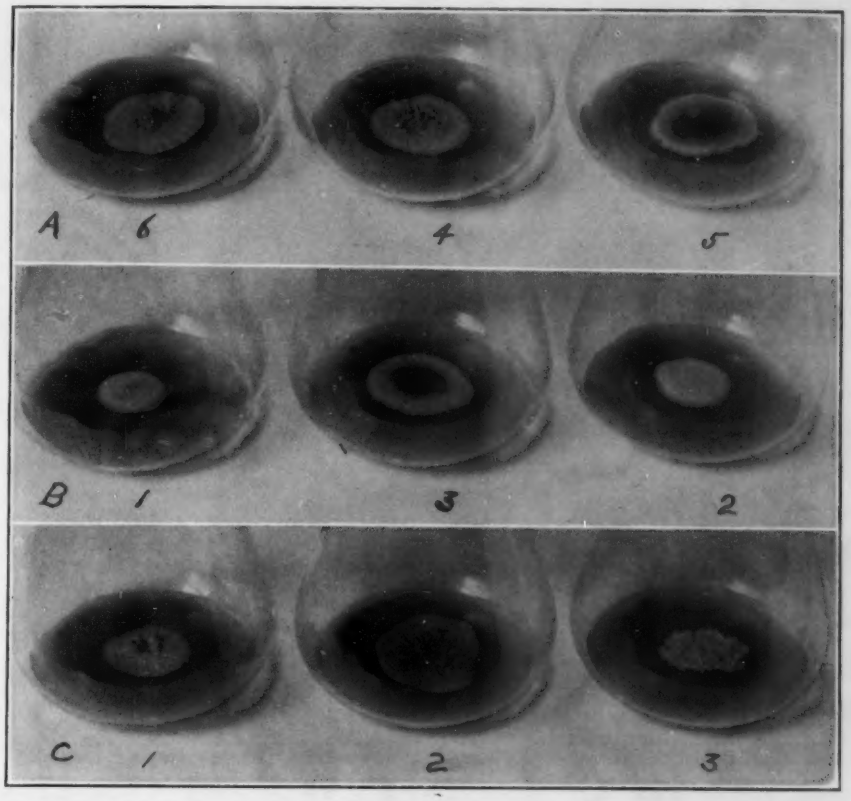

FIG. 1.—Physiologic forms of *Ustilago nuda* and *Ustilago tritici* on potato-dextrose agar, 31 days after inoculation. Series A and B, *Ustilago nuda*, forms 6, 4, 5, 1, 3 and 2. Series C, *Ustilago tritici*, forms 1, 2 and 3.

DISCUSSION

There undoubtedly is physiologic specialization in the loose smut fungi. In fact, *U. nuda* and *U. tritici* are themselves nothing more nor less than physiologic forms. The chlamydospores of the two are almost exactly alike; neither produces sporidia, and the effect on the host plants is essentially the same. The principal difference between them is that *U. nuda* infects barley, whereas *U. tritici* infects wheat. It is true that there are some differences on culture media, but the facts brought out in this paper show clearly that too much significance can not be attached to these differences. If we consider *U. nuda* and *U. tritici* as species, there are at least several physiologic forms within each species. It is significant that some of the forms of *U. tritici* resemble some of *U. nuda* more closely than they do certain forms

TABLE 1.—*Cultural characteristics of physiologic forms of Ustilago nuda on potato-dextrose agar*

Source of collection	Form	Age in days	Size in mm.	Color	Topography	Surface	Aerial mycelium[a]	Margin
Minnesota Writer	1	20	9	Dirty white	Center capitate; ray appressed	Center coarse, felt-like; ray china-like	+ =	Almost entire; myceloid
		31	19	Center becoming brown, grading to gray white	Center convex to pulvinate; ray appressed	do	+	do
Manitoba Writer	2	20	13	Creamy white	Center pulvinate; ray appressed	Long fine felt-like strands	+	Entire; myceloid
		31	23	do	do	Velvet to felt-like	+	do
California F. W. Briggs	3	20	15	Center white, grading to cream	Umbonate and striated	Center of long strands; coarse, felt-like	+ –	Almost entire; myceloid
		31	28	Center peppered with deep brown spots, grading to cream	Umbonate; covered with numerous small tufts; margin appressed	Moist to velvety	+	do
Bulgaria A. Atanasoff	4	20	14	Center cream, grading to white	Raised to convex; smooth center, grading to coarse striations in the ray	Moist to waxy	+ =	Slightly undulating
		31	27	Center tan, grading to white	Raised, ridged and striated counter-clockwise	Waxy to china-like	+ =	Slightly undulating; very slightly myceloid
Belgium E. Marchal	5	20	21	Center cream, grading to dirty white	Convex to umbonate	Center of long strands; coarse, felt-like	+ –	Almost entire; slightly myceloid
		31	28	Center peppered with black tufts, grading to dirty tan	do	Moist to velvety	0	Almost entire; not myceloid

TABLE 1.—(*Continued*)

Source of collection	Form	Age in days	Size in mm.	Color	Topography	Surface	Aerial myceliuma	Margin
Hungary B. Husz	6	20	21	Center tan, grading to white and almost hyaline margin	Radially striated; umbonate	Waxy to china-like	+ =	Undulate; myceloid
		31	30	do	Radially striated; umbonate, with fine rope-like strands	do	0	Undulate; not myceloid

a 0 indicates no aerial myceliun.
+ indicates aerial myceliun abundant.
+ – indicates slight amount of aerial myceliun.
+ = indicates trace of aerial myceliun.

TABLE. 2.—*Cultural characteristics of physiologic forms of Ustilago tritici on potato-dextrose agar*

Source of collection	Form	Age in days	Size in mm.	Color	Topography	Surface	Aerial mycelium[a]	Margin
Minnesota Writer	1	20	14	Dull white	Flat and finely striated	Waxy	0	Undulate to lobate; myceloid
		31	34	Cream center grading to dull white	Flat and finely striated, becoming deeply ridged	do	+ =	Slightly undulate; myceloid
Egypt T. Fahmy	2	20	23	Cream center grading to dull white	Umbonate and coarsely striated	Moist to china-like	+ =	Undulate; myceloid
		31	31	Tan center grading to cream	Raised to convex counterclockwise striations	do	+ =	Slightly undulate; no longer myceloid
New South Wales R. J. Noble	3	20	20	Cream to faint pink	Ribbon-like heaps	Dull china-like	0	Lobate to lacerate
		31	25	do	Ribbon-like heaps raised to 7–8 mm.	do	0	do

[a] 0 indicates no aerial mycelium.
+ = indicates trace of aerial mycelium.

of *U. tritici*. This would seem to indicate that there probably are many forms and that the specialization is not necessarily restricted to wheat or barley. In fact, Humphrey and Tapke (3) have already shown that *U. tritici* can infect rye, and the writer considers it quite probable that some of the forms of *U. tritici* probably will be able to infect barley and vice versa.

While the differences in forms which the writer has demonstrated are cultural and not pathogenic, they are very pronounced and permanent. In fact, the cultural differences between forms of *U. tritici* are sometimes more pronounced than those between so-called *U. tritici* and *U. nuda*. It has been shown clearly that these forms are not mere variants but are constant. Naturally, environmental conditions have a profound effect on the development of the different forms, but under the same conditions they can always be recognized.

Whether these forms will be different pathogenically remains to be seen. It would be rather surprising if they are not. Experiments are now under way to determine this fact.

UNIVERSITY FARM,
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LITERATURE CITED

1. APPEL, O., and E. RIEHM. Die Bekämpfung des Flugbrander von Weizen und Gerste. Arb. K. Biol. Anst. Land u. Forstw. 8³: 24–426. 1911.
2. FARIS, J. A. Physiologic specialization of *Ustilago hordei*. Phytopath. **14**: 537–557. 1924.
3. HUMPHREY, H. B., and V. F. TAPKE. The loose smut of rye (*Ustilago tritici*). Phytopath. **15**: 598–605. 1925.
4. JENSEN, J. L. The propagation and prevention of smuts in oats and barley. Jour. Roy. Agr. Soc. Eng. **24**: 397–415. 1888.
5. REED, G. M. Physiologic races of oat smuts. Amer. Jour. Bot. **11**: 483–492. 1924.
6. SARTORIS, G. B. Studies in the life history and physiology of certain smuts. Amer. Jour. Bot. **11**: 483–492. 1924.
7. STAKMAN, E. C., and J. J. CHRISTENSEN. Physiologic specialization of *Ustilago zeae* and *Puccinia sorghi* and their relation to corn improvement (Abst.). Phytopath. **16**: 84. 1926.
8. TISDALE, W. H., and C. O. JOHNSTON. A study of smut resistance in corn seedlings grown in the greenhouse. Jour. Agr. Res. **32**: 649–668. 1926.

PHYTOPATHOLOGICAL NOTES

Powdery Mildew of Muskmelons in the Imperial Valley of California in 1925.—A large and increasing acreage of early muskmelons (usually known in the West and South as cantaloupes) is grown in the Imperial Valley of California. For 1925 it is estimated that there were 30,000 acres, from which 5,100,000 crates, or 15,000 cars, were shipped to market. The climate of the Valley is extremely arid, with an average annual rainfall of about three inches and a very high percentage of sunshine. Before 1925 there had been no complaint of injury to melon foliage by the numerous fungi and bacteria which attack the foliage in moister climates, and apparently no record of the occurrence of a single organism parasitic on the leaves.

About May 10, 1925, when harvesting was just beginning, local growers and shippers became disturbed over the appearance of powdery mildew, which had apparently never before been observed on muskmelon in the Valley. At that time there were in several of the earlier fields small isolated spots, one to three feet in diameter, where practically all the leaves were dead and drooping, with leaves, petioles, and stems literally covered with the characteristic white to grey powdery growth of mildew. For some distance from the spots isolated, leaves showed young, actively growing mildew. The disease spread rapidly, and by June 6 a high percentage of the older leaves in many of the earlier fields had been killed, giving to the fields a dead-brown appearance. Invariably there was no foliage injury and little or no mildew to be found until melons began to ripen, but in practically every field in the Valley mildew appeared at about that stage and developed rapidly thereafter. The first or main set of melons was not materially reduced in quantity, although it is probable that quality was affected, particularly in the case of later pickings, when many of the older leaves had been killed. By the time the first set of fruits had been harvested the foliage was reduced so much that later set fruits failed to mature. The first set normally makes up a large percentage of the crop in Imperial Valley, and it is generally agreed that the reduction in yield amounted to only about 15 per cent for the whole Valley. It is possible that reduction in quality was of fully as great importance, although neither data nor estimates are obtainable.

Several varieties of muskmelons, including the Honey Dew and other members of the Casaba group, are grown in the Valley, and all were injured by mildew. Various fungicides in both dust and liquid form were applied by the county agent and by several growers, but little or no control of the

mildew was obtained. Failure of the fungicides may have resulted largely or wholly from faulty application, as the mildew was well established before the first applications were made and the machines were not of the most efficient types. Dusting with sulphur was out of the question, as either alone or mixed with hydrated lime it burned the foliage so severely that it was more destructive than the mildew.

At first it seemed that the outbreak might be the result of a rather unusual season, as favorable weather in March and April brought the crop to harvest ten days earlier than ever before, and there were several cool, cloudy days, rather unusual for that season of the year, at the time mildew was first observed. During June, however, sunny weather, high temperature, and low humidity in fully normal degree were the rule. Mildew spread rapidly from the time of its appearance until the end of the harvest in mid-July, with no indications of being influenced by changes in weather. It is probable, therefore, that the fungus was introduced in 1925. The fact that Imperial Valley is surrounded by mountains and desert, with specialized desert vegetation, would account for the absence of the fungus previous to this time.

Perithecia of the fungus have not been found. Consequently it has been impossible definitely to classify it, although *Erysiphe cichoracearum* DeC. usually is found on cucurbits. Failure to find perithecia raises the question of the ability of the parasite to live over to the next crop. In addition to the possibility that perithecia occur rarely, the fungus may be able to persist in vegetative form. Mildew occurred on volunteer melon plants throughout the summer and fall of 1925. Frosts during December and January usually kill all unprotected plants, but on December 30, under the protection of a hedgerow, volunteer plants were found with a few living leaves covered with mildew. Planting of the next crop began early in December under oiled-paper frost protectors, and on December 30 the seedlings showed one or two leaves each, although no mildew was found on them.

The probability of powdery mildew attacking the Imperial Valley melon crop in destructive form each season henceforth is strongly suggested. It is possible, however, that the outbreak of 1925 was of sporadic nature, resulting from some unusual factor or combination of factors, and that nothing similar will occur again for many years.[1]—IVAN C. JAGGER, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[1] In 1926 mildew appeared earlier in the season and was decidedly more destructive, reducing the yield for Imperial Valley from an average of 160 crates per acre to an average of 110 crates.

Black End of Apple.—Observations made on two apple trees at Grady, Montgomery County, Alabama, August 28, 1925, showed a fairly high percentage of the fruits to be affected with the malady described by Barss[1] as "black end." This trouble, though apparently not uncommon on apples and pears in the hot dry sections of Oregon and Washington, impressed the writer as being rather unusual in the Southern States.

The malady is characterized, as shown in figure 1, by a very black discoloration of the skin surrounding the calyx of the fruit. The affected region is circular and measures about 2.5 cm. in diameter. There is a definite margin separating the blackened region from the healthy skin, and the affected area is usually slightly depressed. The flesh is involved only to a very slight extent, but the affected skin and tissue immediately beneath become dry and leathery. As subsequent growth of the fruit occurs, a crack is formed along the margin of the affected area, separating the normal from the abnormal tissue.

Upon first inspection one is liable to confuse black end with arsenical spray injury, due to the similarity of the two disorders. This did not occur in the case under consideration because no spray had been applied. The

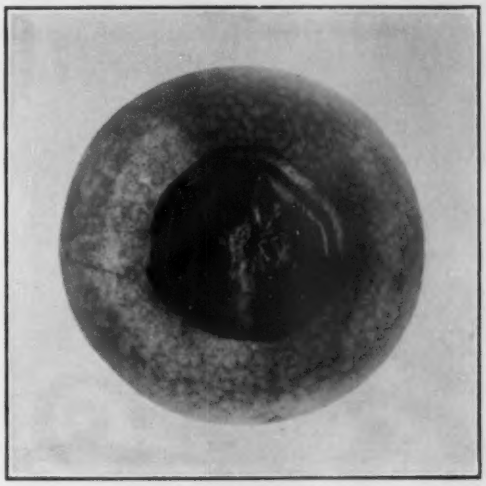

FIG. 1.—A typical case of black end on an apple. x 1.

trouble seemed to be unquestionably the result of the hot dry season, the drought being the most severe in many years. Records show that there was very little rainfall in the locality from February to October. Light rains fell April 4, June 16 and July 18. From this date until October there was

[1] BARSS, H. P. Physiological disorders of developing fruits. Third Crop Pest and Hort. Rept. Ore. Agr. Col. Exp. Sta., 1915–1920. Pp. 159–166. 1921.

no appreciable rainfall. The soil in the orchard was red clay. This no doubt made the drought injury more severe.—S. A. WINGARD, Virginia Agricultural Experiment Station, Blacksburg, Va.

Crown Wart of Alfalfa in the South.—In a recent number of PHYTOPATHOLOGY (Vol. 15, p. 807) the writer reported having found the crown wart of alfalfa near Madison, Indiana. This was thought to be the first report of the occurrence of this disease east of the Rocky Mountains. Further evidence of the distribution of the disease in Eastern United States was obtained in May, 1926, while inspecting alfalfa fields in Mississippi and Alabama. The disease was found in two fields near Muldon and in two fields near Columbus, Mississippi. Later the disease was found both at Uniontown and at Selma, Alabama. Altogether it was found in seven different fields. It is probable that further search would have revealed its presence in still other fields. In most cases a very high percentage of the plants were affected. In many cases the diseased buds either failed to produce stems, or the stems were stunted and sometimes died after they were partly grown. However, in most of the fields the plants were sufficiently thick so that the loss of even a considerable number of stems would not ordinarily be noticed. There was little indication that entire plants were dying, although it was observed that portions of the crowns of some plants apparently were killed by the disease.

Two fields at Uniontown, Alabama, about a mile apart, which were sown at the same time with the same seed had a high percentage of the affected plants. Another field sown at a different date and with other seed and separated from one of the two diseased fields only by a small drainage ditch was apparently free of disease. This points to the possibility that the disease was seedborne. Although the seed dealer is known, no positive proof as to the source of the seed could be obtained.—J. L. WEIMER, Office of Vegetable and Forage Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Kansas Agricultural Experiment Station.

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The History of Seed Disinfection

The Organic Mercuries and New Complex Mixtures Containing Organic Arsenicals

The credit for first developing the organic mercury compounds in application to plants belongs to Friedr. Bayer & Co. in Germany. Prior to 1912, chlor-phenol-mercury had been found effective as a preservative of wood, and possibly the first publication on this compound is that of Falck (Hausschwammforschungen 6: XVI–405, Jena 1912) in connection with his studies on "dry-rot," but for economic reasons it was not developed commercially for this purpose. It was then tested as a seed disinfectant, and the first publication appears to be that of Riehm (Mitt. Biol. Reich. f. Land- u. Forstwirts. 14: 1913), who found chlor-phenol-mercury effective in controlling bunt. His results, as well as those of Remy, Spieckermann, Grevillius, Lüstner and Remy and Vasters, all published in 1914, led to the commercialization of this compound under the name "Uspulun." Since then several hundred articles have been published regarding this organic mercury compound.

The success of Bayer Uspulun led other chemical industries to experiment with organic mercury, and about 1920 Germisan, cyan-cresol-mercury, was brought out by the Saccharin-Fabrik, Aktiengesellschaft, Magdeburg, Germany (Müller & Molz, Deutsch. Landwirts. Presse 48: No. 56. 419. 1921). Another chlor-phenol-mercury compound was brought out in 1924 by E. I. du Pont de Nemours & Company, Inc., under the name "Semesan."

The Bayer laboratories in Germany began investigating organic mercury in dust form in 1916, but it was not until 1925 that Bayer Dust (Uspulun-Trockenbeize), a nitro-phenol-mercury compound, was placed on the market (Heckmanns, Nachrichten d. Landwirts. 4: 12–15. 1925).

One of the most complete reviews of the literature on organic mercuries up to 1922 is the comprehensive paper by Remy and Vasters (Landwirtschaft. Jahrb. 58: 379–480. 1923).

A considerable number of other seed disinfectants have been worked with, but possibly the chief ones deserving serious consideration at the present time are the two products of Farbwerke vorm, Meister, Lucius & Brüning, Höchst am Main. These are complex mixtures containing organic arsenicals combined with mercury or copper, and first designated as Tillantin B and C (Plant, Chem. Zeit. No. 77, 433–437. June 1924; Riehm, Apotheker Zeit. 40: No. 68. Aug. 1925), but now replaced by Tillantin Nassbeize (Anon. Nachrichtenbl. f. deutsch. Pflanzenschutz. 6: No. 9. Sept. 1926) and Höchst Trockenbeize (Riehm, Zeitschr. f. Angew. Chemie 38: No. 46. Nov. 1925; Gesevius & Straib, Deutsch. Landwirts. Presse No. 32. 398–399. Aug. 1926).

C. R. ORTON.

(A series of articles on the subject of SOIL DISINFECTION will begin in the January issue of Phytopathology)

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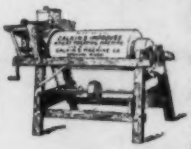

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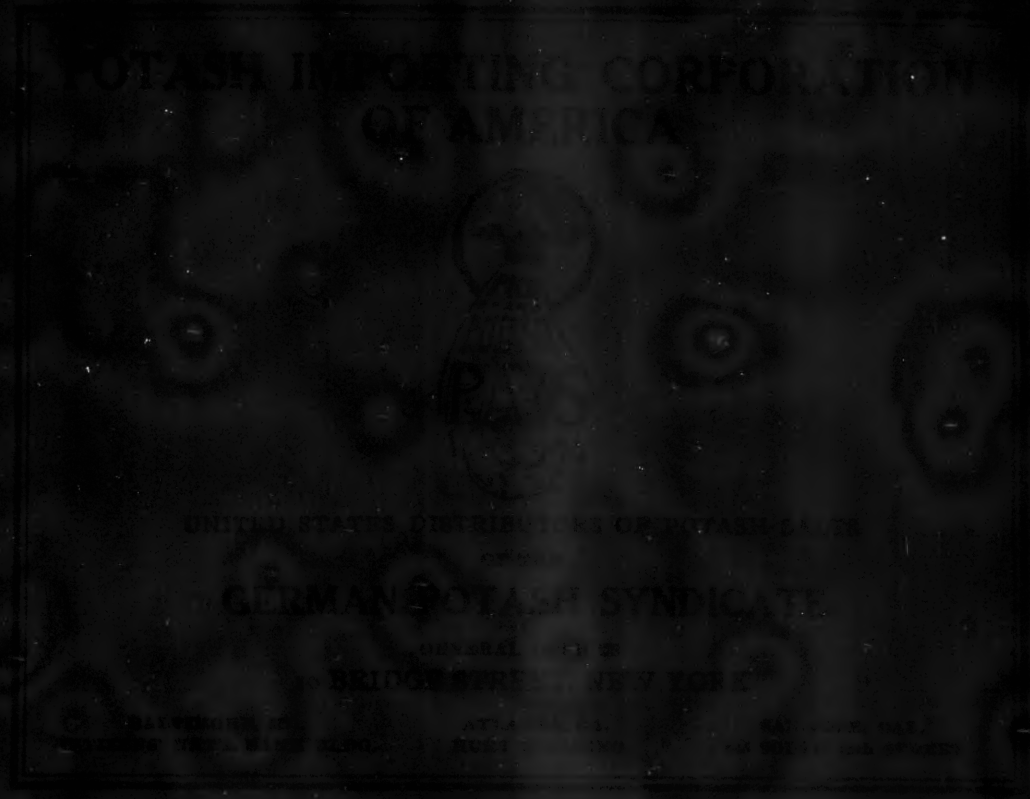
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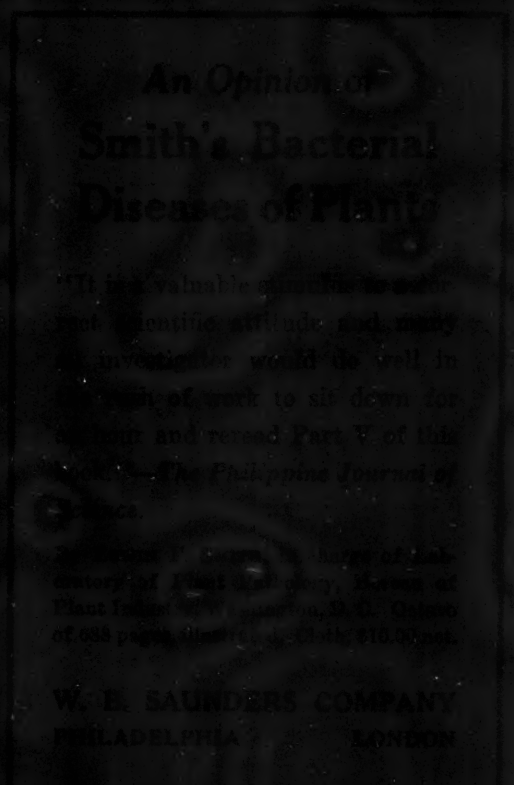
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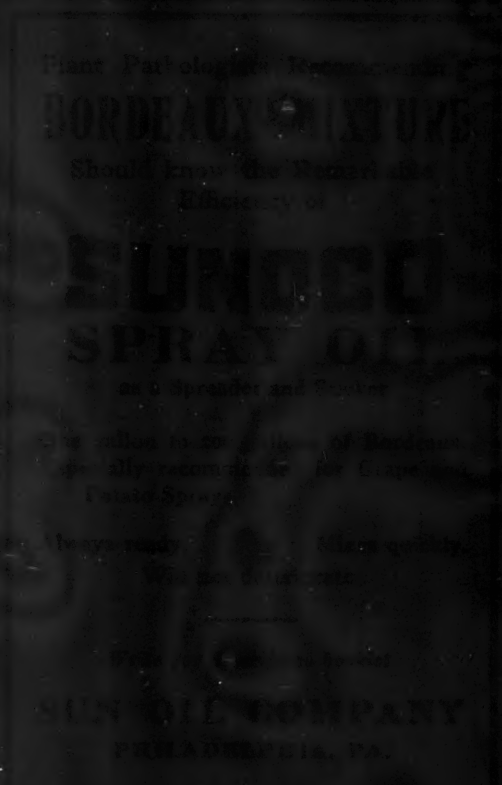
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